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The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

Professional Differences of Fatigue as Revealed by the Method of Electric Flicker.

By

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(Received for publication, December 24, 1948)

The problem of fatigue is of practical importance in our life and one of the urgent questions of labor-hygine and industries at present.

In order to see whether any difference in fatigue according to the kind of business could be found by the method of electric flicker, I made some series of measurements on Japanese railroad men having various kinds of business. The business was classified by common sense into three classes, namely ordinary, a little severe and very severe work.

Experimental.

Method.

I used the same arrangements as described in the work of Motokawa and Suzuki[3] and stimulated the eyes of a subject with rectangular pulses of about 20 cycles per second.

The voltage was increased from zero at a constant rate of 80 mV per second to determine the threshold for the appearance of flicker (S_1), and then the threshold for the disappearance of flicker (S_2) was determined by lowering the voltage from a sufficiently high level at the same rate as above. The difference of the thresholds, S_1-S_2 measured in the morning just before going to work was denoted by ΔS_0, and regarded as the reference value. The difference of both sorts of thresholds, S_1-S_2 obtained during work and recovery was denoted by ΔS, and the value $\Delta S-\Delta S_0$ was taken as a measure of fatigue.

Results.

In Table I are shown the results of ordinary labor (clerk, designer, doctor, and nurse). The data from a designer are represented in Fig. 2 (curve C). The value of $\Delta S-\Delta S_0$ of both subjects, a clerk and a designer rose steadily in the morning, and reached a maximum at 12 a.m. The

descent between 12 a.m. and 1 p.m. was due to the midday recess. The curve rose again because of the work in the afternoon and reached its second maximum at 4 p.m., where the work was over. Since then, the curve descended steadily till the following morning, where the values were found to be −6 mV and −4 mV respectively.

The curves of $\Delta S - \Delta S_0$ for the doctor (subj. No. 3) and the nurse (subj. No. 4) took almost the same course as in the above subjects.

TABLE I.

Ordinary Labor.

No.	Kind of business	Subj. (Age)	Business hours	Time of recess	Degree of maximum fatigue ($\Delta S - \Delta S_0$)	Time of maximum fatigue	ΔS_0 (before work)
1	Clerk	K.W. (20)	8a.m.–4p.m. (8 hours)	1 hour of noon recess	216 mV	4 p.m.	38 mV
2	Desingner of machine	J.U. (27)	Ditto	Ditto	202 mV	Ditto	0 mV
3	Doctor	K.S. (29)	8a.m.–6.30p.m. (10.30 hours)	Ditto	196 mV	12 a.m.	8 mV
4	Nurse	Y.W. (19)	Ditto	No recess for reasons of her business	204 mV	6.30 p.m.	12 mV

The average maximum value of $\Delta S - \Delta S_0$ was 209 mV for this group.

The results of a little severe labor are shown in Table II. The business hours of this group were 24 hours, but the subjects were permitted to take a nap of about 4 hours except locomotive engineers and assistant engineers.

The booking clerks (subj. No. 5 and No. 6) in Table II were far busier in the morning than in the afternoon. The value of fatigue, $\Delta S - \Delta S_0$ rose steadily in the morning, but remained almost constant in the afternoon. At 9.30 p.m. the value of $\Delta S - \Delta S_0$ was found to be 298 mV for the subject No. 5, and 280 mV for the subject No. 6. On the following morning the value of the subject No. 6 was found to be as high as 316 mV, whereas that of the subject No. 5 was found to be only 14 mV. This discrepancy probably rests on the circumstances that the former subject took a nap of only two hours and half, while the latter slept for six hours. Although the value of the subject No. 5 was so low when he got up the recovery of fatigue was not complete, for the value promptly went up to 286 mV by a few hours' work. This instance shows us that a few hours' nap is not sufficient

for a complete recovery from fatigue.

The telegraph operators (No. 7 and No. 8) transmitted and received about 20 telegrams per hour. In the subject No. 7 the value of fatigue rose steadily, and reached 344 mV just before going to bed at 2.30 on the following morning. The value for the subject No. 8 amounted to 242 mV immediately before going to bed at 12 p.m.

The telephone girls (subj. No. 9 and No. 10) took a 10 minutes' rest for every 50 minutes' work without taking any noon recess. The fatigue curves for both subjects took almost the same course. It is interesting to see whether or not the degree of fatigue as measured by our method goes parallel with the quantities of their work represented by the number of exchanges. Therefore both quantities are plotted against the same abscissae in Fig. 1. As can be seen from this figure, both sorts of curves go

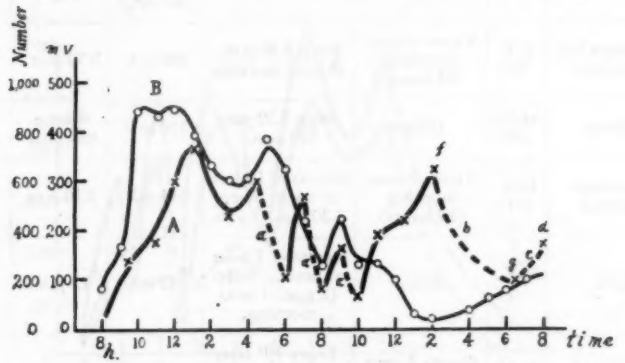

Fig. 1. Relation between degree of fatigue and quantity of work done.
Subj.: telephone girl (see Table II, No. 10).
Abscissa: hours. Ordinate: $\Delta S - \Delta S_0$ (mV) and number of times of exchange.
Curve A: degree of fatigue (mV), Curve B: number of exchanges per hour.
a: time of rest. b: a nap. c: cleaning up of personal effects.
d: time of leaving office. f: end of work. g: getting up.

almost parallel with each other until midnight. The number of telephone calls amounted to about 900 per hour in the morning, and it fell to about 600 per hour during the noon recess of the offices from 12 a.m. to 1 p.m. Corresponding to this change in number of telephone calls the value of fatigue decreased from 300 mV at noon to 228 mV at 3 p.m. As the time for leaving offices drew near the telephone calls increased again to about 800 times per hour and consequently the value $\Delta S - \Delta S_0$ also increased to 292 mV. After 4 p.m. the number of exchanges decreased greatly, and the value of fatigue at 10 p.m. was found to be only 56 mV. The number of exchange decreased further gradually at night, whereas the value of

ΔS–ΔS_0, far from decreasing, rose steadily, as shown in Fig. 1. At 2 o'clock on the following morning the value of ΔS–ΔS_0 increased to 312 mV, though the number of exchanges had fallen to only a few times per hour. The fact that both quantities did not go parallel at midnight is probably due to accumulation of fatigue and sleepiness. Then the value of fatigue fell to 100 mV by a nap of 4 hours until 6 o'clock on the following morning. After having taken enough sleep, the subject showed a small value of fatigue such as 5 mV at 5 p.m.

TABLE II.

A Little Servere Labor.

No.	Kind of business	Subj. (Age)	Business hours	Time of recess	Degree of maximum fatigue (ΔS–ΔS_0)	Time of maximum fatigue	ΔS_0 (before work)
5	Booking-clerk of station	T.T. (24)	8 a.m.–8 next morning (24 hours)	Sleep 9.30 p.m. –4 next morning	298 mV	9.30 p.m.	10 mV
6	Ditto	M.W. (19)	Ditto	Sleep 9.30 p.m. –12 p.m.	316 mV	8 next morning	20 mV
7	Telegraph operator	H.S. (21)	9 a.m.–9 next morning (24 hours)	Recess of 1 hour at noon. Sleep 2.30 a.m.–7 a.m.	344 mV	2.30 a.m.	16 mV
8	Ditto	M.K. (22)	Ditto	Recess of 1 hour at noon. Sleep 12 p.m.–7 next morning.	242 mV	12 p.m.	20 mV
9	Telephone girl	K.Y. (20)	8 a.m.–8 next morning (24 hours)	Every 10′ after each 50′ work. A nap next 2a.m. –630 a.m.	276 mV	7 p.m.	24 mV
10	Ditto	T.A. (20)	Ditto	Ditto	360 mV	1 p.m.	1 mV
11	Locomotive-engineer (only operation on a seat)	N.O. (24)	11 p.m.–next 8.14. a.m.,	Next 2.12 a.m.–3.55 a.m.	316 mV	8.14 next morning.	8 mV
12	Ditto	M.O. (25)	Ditto	Ditto	296 mV	Ditto	1 mV
13	Assistant engineer (burning coal)	Y.A. (29)	Ditto	Ditto	380 mV	Ditto	72 mV
14	Locomotive-engineer	M.S. (28)	8 a.m.–7.40 p.m.	11 a.m.–3 p.m. (4 hours)	212 mV	7.40 p.m.	36 mV
15	Assistant engineer	M.M. (25)	Ditto	Ditto	303 mV	Ditto	40 mV

The values obtained from locomotive engineers and assistant engineers are shown in Table II (subj. No. 11–No. 15). The locomotive engineers were seated while working, so it did not seem that their labor was so hard, whereas the assistants' work, burning coal was much harder one. For that reason, $\Delta S-\Delta S_0$ of all assistant engineers showed higher values than those of all locomotive engineers. The values of fatigue were, of course, found to be greater in long drive than in short one.

For so-called very severe labor, I chose 5 subjects (subj. No. 16–No. 20) from stokers and coolies. The results are shown in Table III. It seemed that the severity of their muscular labor was almost equal. The results from the subj. No. 18 and No. 19 (coolies) are represented by the curves A and B of Fig. 2. The work of the coolies was to load the tender

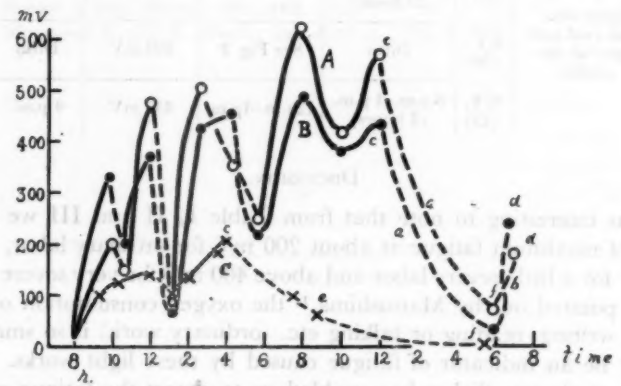

Fig. 2. Fatigue curves of severe and ordinary labor.
Abscissa: hours. ordinate: $\Delta S-\Delta S_0$ (mV).
Curve A: severe labor (subj. Table III, No. 18). Curve B: ditto (subj. Table III, No. 19). Curve C: ordinary labor (subj. Table I, No. 2).
Broken line: at rest. a: a nap. b: cleaning up of personal effects. c: end of work. d: time of leaving office. e: going to bed. f: getting up.

with coal and water. The quantity of work per head for loading coal was 2.5 tons in the morning, 2.7 tons from 1 p.m. to 4 p.m., 3 tons from 5.30 p.m. to 8 p.m., 1 ton from 8 p.m. to 10 p.m. and 4 tons from 10 p.m. to 12 p.m. Both coolies cooperated in loading coal. As can be seen from Fig. 2 (curves A and B), it is to be noted that $\Delta S-\Delta S_0$ rose promptly to a high value at work and fell quickly at rest. This is an interesting contrast in comparison with the curve C of Fig. 2 (ordinary labor; the designer).

TABLE III.

Very Severe Labor.

No.	Kind of business	Subj. (Age)	Business hours	Time of recess	Degree of maximum fatigue ($\Delta S-\Delta S_0$)	Time of maximum fatigue	ΔS_0 (Before work)
16	Storker (burning coal or cleaning an engine in the engine shed)	Y.W. (18)	8 a.m.–4 p.m. (8 hours)	Nearly none.	458 mV	4 p.m.	32 mV
17		S.M. (25)	9 a.m.–7.30 next morning. (24 hours)	Recess of 1 hour at noon. Sleep next 2 a.m.–6 a.m.	635 mV	Next 2 a.m.	20 mV
18	Coolie who loads coal and water on the tender	S.K. (21)	8 a.m.–next 7 a.m. (23 hours)	See Fig. 2	624 mV	8 p.m.	4 mV
19		S.T. (30)	Ditto	See Fig. 2	480 mV	Ditto	20 mV
20		N.T. (25)	8 a.m.–4 p.m. (8 hours)	12a.m.–1p.m.	432 mV	4 p.m.	28 mV

DISCUSSION.

It is interesting to note that from Table I, II and III we can find values of maximum fatigue at about 200 mV for ordinary labor, at about 300 mV for a little severe labor and above 400 mV for very severe labor.

As pointed out by Matsushima,[1] the oxygen consumption or oxygen debt in writing, reading or talking etc. (ordinary work) is so small that it can not be an indicator of fatigue caused by these light works. But the method of electric flicker has enabled us to detect the fatigue caused by the ordinary labor so far studied, and the values obtained have corresponded well to the feeling of fatigue of the subjects. Therefore it may be said that Motokawa's method is valuable especially for detecting light degrees of fatigue.

Bogoslovsky[2] reported that by visual exertions the electric sensitivity of the eyes decreases and recovers after cessation of visual work. But from our findings it is clear that our measure $\Delta S-\Delta S_0$ indicates not only the fatigue of the eyes but also that of the whole body, because the fatigue caused by muscular work can be detected by our method more easily than that caused by visual exertion.

SUMMARY.

By making use of the method of electric flicker, I measured throughout 24 hours the degree of fatigue and recovery of Japanese railroad men in all kinds of labor (ordinary, a little severe and very severe labor).

The degree of fatigue caused by ordinary labor amounted to about 200 mV when they were leaving office, the maximum value for a little severe labor was about 300 mV at the end of work, and that for very severe labor rose above 400 mV.

The value of fatigue measured by Motokawa's method of electric flicker run nearly parallel with the quantity of work done, and corresponded well to the felling of fatigue in many cases.

The author has pleasure in acknowledging his indebtedness to Prof. K. MOTOKAWA who has guided him kindly.

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The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

Studies on the Antibiotic Substances from Actinomyces.

(5th Report)

„ Secondary Screening " Test on 15 Strains of Actinomyces, Specifically Antagonistic to Acid-fast Bacteria.

By

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(Received for publication, December 26, 1949)

As previously reported many studies on streptomycin and streptothricin-like substances has been conducted in this laboratory.

The present paper deals with 15 strains producing the antibiotics specifically active on acid-fast bacteria only (IV group of actinomyces).

After many experiments antibiotic activities were proved in their culture filtrates of 11 out of 15 strains of actinomyces, using several kinds of culture media by surface and shaking culture methods. Modified Waksman's media containing 1–2 per cent glycerol and used in surface culture method were found most suitable in order to obtaine a high potency of such a antibiotic substance.

Though the activities of culture filtrates were not very powerful in general, they corresponded almost with those of streak plate method (so-called " primary screening "). So the culture filtrates of almost all strains of this group of actinomyces were not inhibitory to gram-positive and negative organisms and exclusively active on acid-fast bacteria, using My. phlei, My. smegmatis and My. tuberculosis as test organisms.

Some strains, however, showed such a specificity only in " primary screening ", and in " secondary screening " they were proved to be somewhat inhibitory to non acid-fast organisms too. In the latter case, B. anthracis was often more sensitive than the others, as already anticipated in " primary screening ".

Properties of active filtrates were somewhat different from one another, showing that all of them may produce unknown specific antibiotic substances respectively. A new antibiotic substance specifically active on My. phlei was isolated and crystallized by Ouchi from the culture filtrates of Str. No. 364, one of most active strains of this group.

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Oxygen Consumption during and after Exercise and its Relation to the Degree of Fatigue as Measured by the Method of Electric Flicker.

By

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(Received for publication, December 24, 1948)

Motokawa and Suzuki[10] have recently reported that a flicker sensation caused by electric stimulation of the eye can be used as a very convenient indicator for the measurement of fatigue. They used rectangular pulses of 20 cycles per second as stimulation. The stimulus voltage was increased from zero at a constant rate of 80 mV per second to determine the threshold S_1 for the appearance of flicker, and then the threshold S_2 for the disappearance was determined by lowering the voltage from a sufficiently high level at the same rate as above. The difference of the thresholds S_1–S_2 at rest was chosen as the standard value and denoted by ΔS_0. The difference S_1–S_2 obtained in a fatigued state or in the recovery course was denoted by ΔS. It was shown with many examples that the difference ΔS–ΔS_0 can faithfully indicate the degree of fatigue. Moreover they remarked that the above method of electric flicker is very sensitive to oxygen insufficiency of the body. This fact suggests that oxygen lack in the body plays some important rôle at least in some kind of fatigue.

Hill, Long and Lupton[2] established a connection between fatigue and oxydation in the body or oxygen consumption, and more detailed measurements of gas-metabolism have been made in connection with fatigue by a number of investigators such as Pembrey and Cook,[1] Best, Furusawa and Ridout,[5] Tomohisa, Sasaki and Matsuyama[8-9] and others.

In order to investigate the relation between the degree of fatigue (ΔS–ΔS_0) measured by the method of electric flicker and the oxygen consumption in the body, I have made the following experiments for three kinds of exercise.

Experimental.

Method.

For 30 minutes before exercises, the subjects were kept seated quietly.

A series of measurements was taken after a rest to obtain the standard value, and then similar measurements were made with intervals of about 5 minutes during exercise as well as in the recovery course. a) I chose two subjects for the exercise by a bicycle ergometer. The load was 120 kgr m per min. and the duration of exercise was 45 minutes. b) I chose two subjects and made them run 550 meters at their top speed. After the running I pursued the recovery for 50 minutes by means of the electric method and of measurements of oxygen consumption. c) I chose a subject, and made him ascend and descend the stairs several times. The height of the stairs was 5 meters. The stairs consisted of 22 steps. The inclination angle was 38°. The subject went up and down the staris at a rate of 2 times per minutes.

The electrical method used was the same as in the previous work.[10] The stimulating electrodes were placed at the glavella and the occiput. Immediately before exercise I measured the thresbold for appearence of flicker (S_1) and that for disappearence of flicker (S_2) in order to evaluate the difference ΔS_0. The difference of thresholds S_1 and S_2 during exercise and recovery was denoted by ΔS, and the difference $\Delta S - \Delta S_0$ was taken as a measure of fatigue as in the previous work.

In order to measure oxygen consumption, I gathered the expired air of a subject through the hose from the mouthpiece into a bag holding about 70 litres. At time of rest and at a later stage of recovery I collected the gas for 5 minutes, but for 2 or 3 minutes during the actual exercise or at early stages of recovery. A part of the gas collected was poured immediately into a sampling-tube (50 c.c.) for the gas analysis, and the value of the gas left in the bag was measured by a sprirometer. The gas was analysed by means of Haldene's apparatus. The oxygen consumption per minute at time of rest was expressed by O_0, and that during exercise and in the recovery course by O in order that the difference $O - O_0$ might be compared with our measure of fatigue $\Delta S - \Delta S_0$.

Results.

a) Exercise: Bicycle ergometer. Subject: Ki. S. The values of S_1 and S_2 are plotted as ordinates against minutes as abscissae in Fig. 1. At rest S_1 and S_2 were 480 mV and 476 mV respectively, the difference ΔS_0 being only 4 mV. One may think that the values of thresholds S_1 and S_2 would always increase by fatigue, but this is not always the case, as shown in this example; the values of thresholds decreased probably as the result of sweating which lowered the skin resistance and consequently increased the current intensity. To be sure that the sweating actually decreases the thresholds I applied a wet towel warmed to 50°C to the forehead of the subject for 5 minutes in order to provoke local sweating and

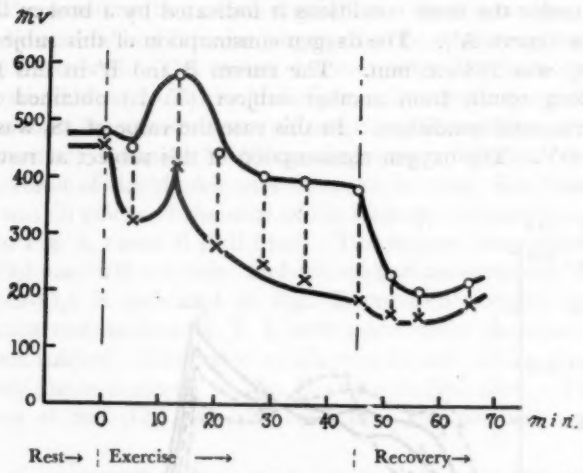

Fig. 1. Changes of threshold for appearance of flicker S_1 and that for disappearance of flicker S_2 during exercise by a bicycle ergometer.
Subj.: Ki.S. (29 years old). Abscissa: time.
Ordinate: thresholds of electric flicker.
Circle: S_1. Cross: S_2. Broken line: S_1–S_2. (see Fig. 2).

measured both sorts of threshold. The data obtained under this condition and in the usual non-sweating state are compared in Table I. As seen from this Table, there is a great difference between the thresholds at sweating

TABLE I.

Difference of Absolute Thresholds Measured in Sweating-time and Non-sweating-time at Rest.

(Local sweating was provoked by application of a warmed towel on the forehead for 5 minutes.)

Subj.	Non-sweating-time			Sweating-time		
	S_1	S_2	S_1–S_2	S_1	S_2	S_1–S_2
T. Ito	330 mV	312 mV	18 mV	166 mV	156 mV	10 mV
Ki. Suzuki	260 mV	254 mV	6 mV	126 mV	116 mV	10 mV

time and at non-sweating time. But the values of S_1–S_2 are not so different in both cases. This fact shows that sweating has little influence on our measure of fatigue, though it affects greatly the absolute value of thresholds.

The values of ΔS–ΔS_0 of Fig. 1 are expressed with a full line in Fig. 2 (curve A). The oxygen consumption per minute (O–O_0) which was

measured under the same conditions is indicated by a broken line in the same figure (curve A'). The oxygen consumption of this subject at time of rest (O_0) was 243 c.c./min. The curves B and B' in this figure are corresponding results from another subject (T. I.) obtained under the same experimental conditions. In this case the value of ΔS_0 was found to be only 4 mV. The oxygen consumption of this subject at rest (O_0) was 248 c.c./min.

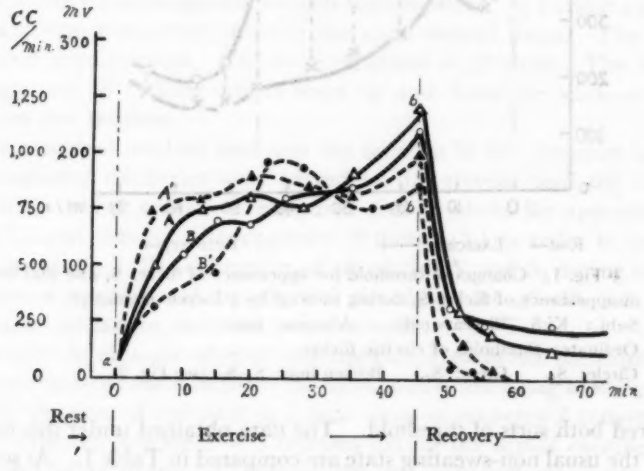

Fig. 2. Fatigue and recovery curves for exercise by a bicycle ergometer.
Abscissa: time of exercise and recovery.
Ordinate: $\Delta S-\Delta S_0$ (mV). and oxygen consumption (c.c./min.)
Curve A, A': subj. Ki. S. Curve B, B': subj. T.I. (20 years old).
Full line A, B: $\Delta S-\Delta S_0$ (mV). Broken line A', B': oxygen consumption (c.c./min.).
a: beginning of exercise. b: end of exercise.

The above four curves obtained from two subjects seem to be passing into the " second wind " about 10–15 minutes after the beginning of exercise, and then preserve a form of " steady state " for about 25 minutes. The subjects appeared to be using chiefly the muscules under the waist during this time, but after about 40–45 minutes the muscules above the waist obviously took part in the exercise. It can be seen from the curves that oxygen consumption and $\Delta S-\Delta S_0$ gradually increase in this states from the plateau attained after the second wind. In the course of the exercise the values of $\Delta S-\Delta S_0$ and $O-O_0$ run almost completely parallel. However, no parallel going is seen in the recovery course; after the end of the exercise the oxygen consumption of the two subjects recovered almost completely in about 10 minutes, while the value of $\Delta S-\Delta S_0$ rapidly ap-

proached the zero-line but never reached it. In this manner fatigue as measured by our method remains longer after the oxygen consumption has completely recovered. This point deserves attention because it means that our method is more sensitive to fatigue and more adequate for measuring fatigue than the measurement of oxygen consumption.

b) Exercise: Running. 550 m. Top speed. Subjects: Ka. S. and T.I. The result of the electric measurement for subj. Ka. S. during the rest (ΔS_0) was 66 mV, and the state of his recovery after running ($\Delta S - \Delta S_0$) is shown in Fig. 3, curve B (full line). The oxygen consumption during the rest (O_0) was 105 c.c./min. and his oxygen consumption during the recovery ($O - O_0$) is indicated in Fig. 3, curve B′ (broken line). The electric measurements for subj. T. I. were taken under the same conditions as the above subject. The value of ΔS_0 was 12 mV at the time of rest. His recovery curve is shown in Fig. 3, curve A (full line). The oxygen consumption at rest (O_0) was 220 c.c./min. The recovery curve of the

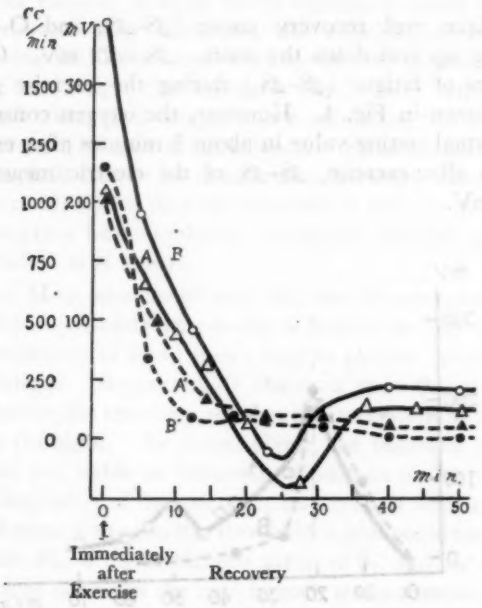

Fig. 3. Recovery curves after running (550 m. top speed).
Abscissa: time of recovery after exercise.
Ordinate: $\Delta S - \Delta S_0$ (mV) and oxygen consumption (c.c./min.).
Curve A, A′: subj. T.I. (20 years old). Curve B, B′: subj. Ka. S. (22 years old).
Full line A, A′: $\Delta S - \Delta S_0$ (mV). Broken line: oxygen consumption (c.c./min.).

oxygen consumption after the running is plotted in Fig. 3, curve A′ (broken line).

The above mentioned four curves of the two subjects show the following facts.: The oxygen consumption of each subject has almost recovered in 10 or 15 minutes immediately after exercise. The curves of $\Delta S-\Delta S_0$ for both subjects went down to the negative phase in about 25 minutes after exercise and went up again. In 50 minutes after running, the electric values have not yet completely recovered.

It is an interesting fact that there is a negative phase in the recovery course of $\Delta S-\Delta S_0$. In my experiments on oxygen consumption I could find no such negative phase, but Hill, Long and Lupton[3] reported that the respiratory quotient falls to lower values than its normal resting values about 30 minutes after severe exercise. Tomohisa, Sasaki and Matsuyama[8] also reported similar results. Such a negative phase appeared always after severe exercise when the recovery was pursued by our electrical method.

c) The fatigue and recovery curve ($\Delta S-\Delta S_0$ and $O-O_0$) for the exercise of going up and down the stairs. $\Delta S_0=22$ mV. $O_0=229$ c.c./min. The degree of fatigue ($\Delta S-\Delta S_0$) during the exercise goes parallel with $O-O_0$ as shown in Fig. 4. However, the oxygen consumption falls almost to the normal resting value in about 5 minutes after exercise, while even 50 minutes after exercise, $\Delta S-\Delta S_0$ of the electric measurement still remains at 22 mV.

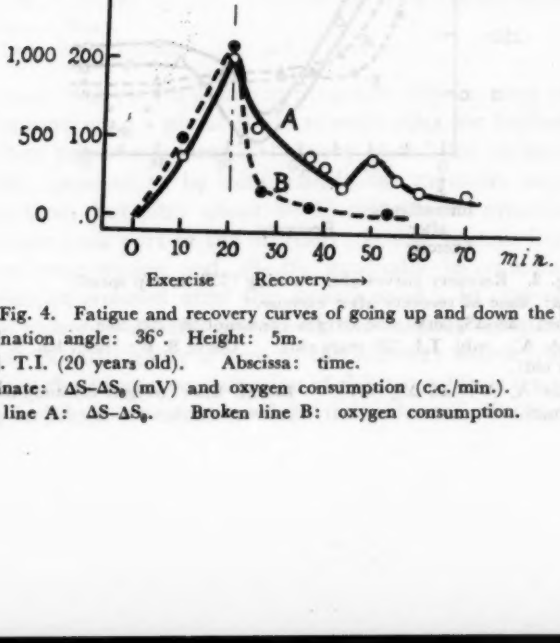

Fig. 4. Fatigue and recovery curves of going up and down the stairs.
Inclination angle: 36°. Height: 5m.
Subj. T.I. (20 years old). Abscissa: time.
Ordinate: $\Delta S-\Delta S_0$ (mV) and oxygen consumption (c.c./min.).
Full line A: $\Delta S-\Delta S_0$. Broken line B: oxygen consumption.

Discussion.

With regard to the relationship between the electrical measurements and oxygen consumption the following facts are remarkable. In the exercise by a bicycle ergometer (moderate exercise), both subjects came into their second wind 10 or 15 minutes after beginning of exercise, and their oxygen consumption showed a steady state. After 40 or 45 minutes this steady state was broken by the increase in number of muscles taking part in the exercise. This characteristic course was found also in the curve of electric measurements. Also, in the exercise of going up and down the stairs, the parallel relationship between $\Delta S - \Delta S_0$ and $O - O_0$ was found. At least during exercise the value of $\Delta S - \Delta S_0$ runs nearly parallel with the oxygen requirement in the body.

As to the recovery the following facts may be worth mentioning. In the exercise by a bicycle ergometer, the oxygen consumption has almost recovered within 5–10 minutes after exercise, while in the case of running, which is severer exercise, it takes 10–15 minutes to reach the same state of recovery as above. This resembles the experiments of Hill, Long and Lupton[3] showing that the severer the exercise, the greater the oxygen debt and therefore the longer the recovery time. It is remarkable that the value of $\Delta S - \Delta S_0$ remained longer above zero than the oxygen consumption in the recovery course of all subjects. Embden and Jost[4] said that one can not explain fatigue by the lactic acid theory which was advanced by Hill. My result shows that the recovery is not yet complete when the oxygen consumption has completely recovered, and this fact scarcely conforms to the lactic acid theory.

Kaem and Mogendowitsch[6] said that the measurement of the electric sensitivity of eyes (absolute threshold) is helpful to the study of fatigue, because the sensitivity of blind men's eyes to electric currents decreases as the result of fatigue. Bogoslovsky[7] observed that the electric sensitivity of the eyes is low in the morning, reaches the maximum at 12 a.m. and goes down slowly in the night. As stated above, the values of electrical thresholds themselves are liable to influences of various unessential factors such as sweating, adaptation of the eye etc., and are not adequate as the index of fatigue. Moreover the electric threshold is less sensitive to fatigue than our measure $\Delta S - \Delta S_0$, if the arithmetic mean of S_1 and S_2 is taken as the threshold value as is always the case in such measurements. However the question why $\Delta S - \Delta S_0$ can be a faithful measure of fatigue is still open and awaits further studies.

Summary.

In exercise by a bicycle ergometer, running and exercise of going up

and down stairs, I compared the oxygen consumption with the degree of fatigue as measured by the method of electric flicker and obtained the following results.

Data of the electrical measurements went nearly parallel with oxygen consumption during the exercises, while during the time of recovery some discrepancy was found between both sorts of measurements; the recovery was attained always earlier as to oxygen consumption than as to the electrical values.

The time course of recovery showed sometimes especially after severe exercise a negative phase which could be proved more clearly by the electrical method than by the measurement of oxygen consumption.

The author has pleasure in acknowledging his indebtedness to Prof. K. Motokawa who has guided him kindly. He also acknowledges with gratitude the support given to him by Prof. K. Matsuda.

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The Blood Group Substance in Red Blood Cells.

First Report.

A Search for the Group (A, B, O)-Specific Substance in the Lipoid Fragment.*

By

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Most of the claims that a group-specific lipoid is present in erythrocytes were based on demonstration of the group activity in the fractions extracted either by alcohol or by ether, and Euler and Molitsch, the only farther-going investigators, pointed to no more than the precipitability of the substance from ether by acetone. The chemical nature of the active factors (agglutinogens) in erythrocytes has been thus entirely obscure. Exhaustive fractionation, which will be accounted for below, disclosed that they are a substance well soluble in hot water and containing psychosin and phosphorus. In addition a "co-adsorbent" common to all the groups of erythrocytes was obtained thereby. This was found similarly composed as the above group substances qualitatively and raised the anti-agglutinative potency of the latter.

Experimental.

Separation of the Group Substances and Co-adsorbent.

It was carried out by taking the anti-isohemoagglutinative potency of fractions as an indication.

1. Preliminary fractionation of the lipoid fragment of erythrocytes.

Red blood cells were collected by centrifuging 3 *l.* of A group blood, washed with physiological saline repeatedly, until a new washing gave no biuret reaction, and placed in 5 volumes of 94% alcohol. After the lapse of 1 week, the mixture was centrifuged and the centrifugate dried in a

* 2nd report of Masamune and co-workers' "Chemistry and Biology of Lipoids".

desiccator (H_2SO_4). It was then treated with 1 *l.* of boiling 94% alcohol four times (5, 3, 2 and 2 hours) (the residue named R). The cold and hot alcohol extracts were distilled together under reduced pressure to dryness and the remainder treated with ether (in total 50 cc.) thoroughly. The fraction taken up in ether (the ether-soluble and -insoluble fractions named Fr. Es and Fr. En respectively) was dried up by evaporating off the solvent and fractioned with acetone (two 40 cc. portions) (Frs. Acs (=acetone soluble) and Acn (=acetone insoluble)) and the acetone-insoluble part further divided into the benzene soluble and insoluble fractions (50 cc. of benzene were used) (Frs. Bzs and Bzn). Fr. Bzs was boiled with 30 cc. of acetone and the undissolved residue (the fraction dissolved—Fr. Achs—was put aside for the treatment below) was washed with cold acetone (three 20 cc. portions). And after drying in a desiccator, it was dissolved with 15 cc. petroleum ether three times (Fr. Pes) (only a small portion (Fr. Pen) did not go into solution) and after again drying, washed with three 50 cc. portions of absolute alcohol. The rest was dried up—Crude A-S.S. I. Yield, 266 mgm. It inhibited isoagglutination of A erythrocytes at a dilution of 1: 2000 (regarding the technique see below).

Now returning to Fr. En, this was stirred up with 30 cc. of tetralin in a bath at 60°C and centrifuged while hot. The centrifugate was treated likewise with tetralin again and again, until no clouring matter came out. The extracts (250 cc. altogether) were precipitated with 3 volumes of acetone and after standing in an ice-chest overnight, the precipitates were collected by centrifugation (no precipitation occurred on further addition of acetone to the mother liquid) and washed well with acetone and dried. The obtained brown-gray powder amounted to 160 mgm.—Crude A-S.S. II.

Frs. Acs and Achs above were taken up in 20 cc. of benzene, into which most of them passed over (the insoluble part was washed with benzene and dried by means of a jet pump), and distilled under diminished pressure to dryness and then treated with petroleum ether (20 cc. in total). The insoluble substance here was washed with petroleum ether and dried—Crude Co-adsorbent, (120 mgm.). From the extracts came out on evaporation an abundant number of crystals which had the appearance of cholesterol. The whole evaporated residue is designated Fr. Acs-pes.

2. Purification of the crude products.

It was tried to further purify Crude A-S.S.'s and Crude Co-adsorbent by partition between two solvents.

Crude A-S.S. I. The substance was dissolved in 160 cc. of 0.9% NaCl solution (extracted many times with small portions of the salt solution amounting to 160 cc. in total), alkalized with 0.1 n. NaOH to pH 9.6 after having removed a small insoluble part (about 10 mgm.), and shaken

twice with 100 cc. of benzene in a separatory funnel. The watery layer was separated, removed from the dissolved benzene by replacing this with ether, which was expelled off by aeration, neutralized with 5% acetic acid, and distilled at a low temperature to about 10 cc. To the condensate were added 180 cc. of acetone, whereupon flocculent precipitates appeared. The precipitates were centrifuged and washed with three 30 cc. portions of 97% alcohol and dried in a desiccator.

The powder obtained was taken up in 80 cc. of tetralin (the tetralin-insoluble part consisted mostly of ash and hence freed therefrom by repeated washing with alcohol (94%)—Fr. Tn), shaken violently with 200 cc. of 0.01 n. NaOH and 500 cc. of ether and stood. The brownish watery layer was separated and combined with the washings of the organic mixture, neutralized with 0.1 n. HCl and distilled in vacuo to some 10 cc.

Followingly the condensate was acidified to pH 2.0 with hydrochloric acid and shaken with 30 cc. portions of chloroform as far as a new change of the latter got coloured brownish. The chloroform extracts were washed together with water over and over again to eliminate HCl completely and dried up by vacuum distillation—Fr. Chs. The yellowish fluid exhausted with chloroform and the washings of the extracts were combined, neutralized and distilled in like manner to about 2 cc. and the deposit after addition of 20 cc. of absolute alcohol washed with alcohol many times and dried (Fr. Chn I). Fr. Chs was washed repeatedly with acetone (four 20 cc. portions) and dried again—A-S.S. I (50 mgm.). The washings here were also distilled to dryness (about 10 mgm.).

A-S.S. I was inhibitory to isoagglutination of A erythrocytes at a dilution of $1:2\times10^4$.

Crude A-S.S. II. This was subjected only to partition between water and chloroform, namely:—It was emulsified in 30 cc. of water, acidified to pH 1.0 by the aid of 0.1 n. HCl (15 cc.) and extracted with 30 cc. portions of chloroform as before. The part that remained in water was recovered by distilling the neutralized (0.1 n. NaOH was used) solution, and washed with acetone (two 30 cc. portions). The acetone-insoluble fraction was dried—Fr. Chn II—and the acetone-soluble separated from the solvent by evaporation and dried. On the other hand, the chloroform extracts united (100 cc. in total) were washed with three 50 cc. portions of water and distilled under reduced pressure, and the remainder washed with 30 cc. of acetone and dried. The brownish gray powder yielded was 21 mgm.—A-S.S. II.

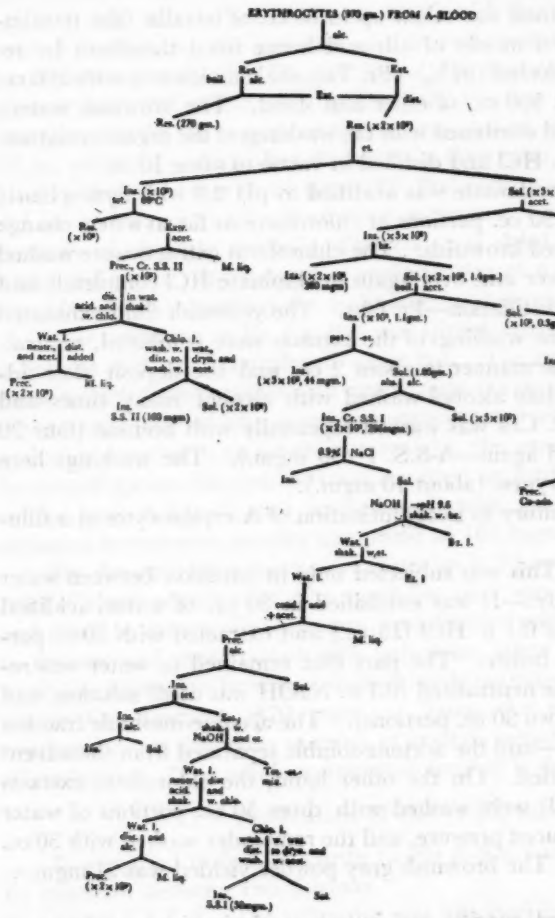
A-S.S. II exhibited specific anti-isohemoagglutinative activity until $1:10^5$ dilution.

Crude Co-adsorbent. It was dissolved in 4.5 cc. of boiling acetone and filtered immediately. When the filtrate became cold, precipitates appeared.

TABLE I.

Fractionation of the Lipoid Fragment of A Erythrocytes.

(In parentheses are given the weights of the fractions and their maximum dilutions, at which isohemoagglutination was inhibited. acet.=acetone. alc.=alcohol. bz.=benzene. chlo.=chloroform. dis.=dissolved. dist.=distilled. et.=ether. l.=layer. liq.=liquid. m.=mother. petr.-et.=petroleum ether. tet.=tetralin. wat.=water. ina.=inactive.)



These were centrifuged and washed with 4.5 cc. of boiling acetone (this time no dissolution occurred) and with cold acetone and dried. Yield, 30 mgm.—Co-adsorbent. This substance strengthened the group lipoids in inhibiting the erythrocyte iso-agglutination as will be seen in Table II, whereas it was devoid per se of the anti-agglutinative force.

The weights and anti-agglutinative activities of the intermediate fractions are appended in Table I

From 2500 cc. of B blood, in a like way, were prepared 46, 3 and 10 mgm. respectively of B-S.S.'s I and II and Co-adsorbent. The crops of the corresponding fractions from 200 cc. of O blood were 5, 1 and 2 mgm.

Serological, Physical and Chemical Properties of the Products.

1. The anti-isohemoagglutinative potency.

Original solutions or emulsions for the measurement were prepared by adding to 1–10 mgm. of the fractions 0.5 cc. of 0.01 n. NaOH at first and afterwards 0.5 cc. of 1.7% saline solution. They were progressively diluted with physiological saline to follow our usual technique. The effect of Co-adsorbents was examined by addition of 0.05 cc. of their 0.5% solutions to 0.2 cc. of the above solutions or emulsions, original as well as diluted. Accordingly the dilution of the antiagglutinative fractions was increased 2.5/2 times in this case, but it was not taken into account in calculating out their maximum effective dilutions given in the tables (Tables I and II). Of the results, important some are illustrated in detail in Table II. All of the employed A and B sera had agglutinin titre 160. In the controls where the use of the fractions was omitted, typical agglutination of the erythrocytes occurred by the antisera, but no agglutination at all when serum was replaced with physiological saline besides.

As is seen from Table II, Co-adsorbents are non-anti-isohemoagglutinative intrinsically and activate group lipoids irrespective of the red cells wherefrom they had been separated, in other words, one and the same co-adsorbent looks obtainable from A, B and O cells. The writer also noticed it to promote the seroreaction of the group carbohydrate separated from the mucus-mucin of pig stomach.

2. Solubility of the final products.

Similar solubility was shown by the final products from the erythrocytes belonging to the same blood group as is illustrated in Table III. The substances from A erythrocytes were less soluble in organic solvents than those from B erythrocytes. As will be mentioned in Discussion, the

TABLE II.

Occurrence of Agglutination of Erythrocytes by Normal Antisera Treated with a Group-specific Lipoid-fraction from Erythrocytes or with a Co-adsorbent-fraction or with a Group-specific Lipoid-fraction and a Co-adsorbent-fraction.

(– no agglutination, ± faint agglutination, + slight agglutination, ++ marked agglutination, +++ strong agglutination.)

Substance	Group of serum	Dilution of group-specif. fractions.											
		1% (Original)	×2	×5	×10	×2×10	×5×10	×10²	×2×10²	×5×10²	×10³	×2×10³	×5×10³
Crude A–S.S.I	B	–	–	–	–	–	+	+	++	+++	+++	+++	+++
	A	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Crude A–S.S.I + Co-ads. fr. A cells	B	–	–	–	–	–	–	–	–	+	++	+++	+++
Crude A–S.S. II	B	–	–	–	–	–	++	+++	+++	+++	+++	+++	+++
	A	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Crude A–S.S.II+ Co-ads. fr. A cells	B	–	–	–	–	–	–	–	+	++	+++	+++	+++
Crude A–S.S.II+ Co-ads. fr. B cells	B	–	–	–	–	–	–	–	–	+	++	+++	+++
Crude A–S.S.II+ Co-ads. fr. O cells	B	–	–	–	–	–	–	–	–	+	++	+++	+++
Crude B–S.S. I	B	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	A	–	–	–	–	+	++	+++	+++	+++	+++	+++	+++
Crude B–S.S.I+ Co-ads. fr. A cells	A	–	–	–	–	–	–	–	++	+++	+++	+++	+++
Crude B–S.S. II	A	–	–	–	–	+	++	+++	+++	+++	+++	+++	+++
Crude B–S.S.II+ Co-ads. fr. A cells	A	–	–	–	–	–	–	+	++	+++	+++	+++	+++
A–S.S.I	B	–	–	–	–	–	–	–	–	++	++	+++	+++
A–S.S.I + Co-ads. fr. A cells	B	–	–	–	–	–	–	–	–	–	+	++	+++
A–S.S.II	B	–	–	–	–	–	–	–	–	–	–	++	+++
B–S.S.I	A	–	–	–	–	–	–	+	++	+++	+++	+++	+++
B–S.S.II	A	–	–	–	–	–	–	+	++	+++	+++	+++	+++
Co-ads. fr. A cells	B	±	+	++	+++								
	A	+++	+++	+++	+++								
Co-ads. fr. B cells	B	+++	+++	+++	+++								
	A	+	++	+++	+++								
Co-ads. fr. O cells	B	+++	+++	+++	+++								
	A	+++	+++	+++	+++								

latter were probably not so pure as the former.

S.S.'s dissolved readily in alkaline water (NaOH solution, pH 9.6) in the cold and acidification (with HCl) to pH 2.0 precipitated the solutions of A-S.S.I and B-S.S. II but caused those of the other S.S.'s only turbidity. All gave a homogeneous solution in 0.9% saline and hot water and proved atypical lipoids.

In general the solubility was reversible between low and high temperatures.

TABLE III.

Solubility of S.S.'s I and II* and Co-adsorbent from A-erythrocytes.*

(− insoluble, ± faintly sol., + partly sol., ++ mostly sol., ### completely sol. "hot" signifies that the solvents were heated just to boiling.)

Solvent \ Substance		A-erythrocytes		B-erythrocytes		Co-adsorbent from A-erythrocytes
		S.S. I	S.S. II	S.S. I	S.S. II	
Water	cold	emulsifies	emulsifies	emulsifies	opalescent sol.	−
	hot	###	###	opalescent sol.	###	++
Methanol	cold	−	−	++	+	
	hot	−~±	+	###	++	
Ethanol (abs.)	cold	±~+	±~+	++	−	−
	hot	+	+	###	+	−
Ether		−	−	−	###	−
Petroleum ether		−	−	++	++	−
Acetone	cold	−	−	−	−	−
	hot	−	−	++	++	−
Chloroform		###	++	###	###	###
Benzene	cold	###	++	###	###	###
	hot	###	###	###	###	###
Pyridine		###	###	###	###	
Tetralin	cold	###	###	###	###	###
	60°C	###	###	###	###	###
Gl. acetic acid	cold	+	+	−	−	
	hot	###	++	###	###	

* O-S.S.'s and Co-adsorbents from B and O erythrocytes could not be examined in the present respect owing to shortage of material.

3. The sequels of qualitative tests.

Qualitatively the substances resembled one another. They gave positive Molisch (for ordinary sugar) and Thudicum (for psychosin) reactions. Not only were biuret and ninhydrin reaction not given, but methylpentose (Rosenthaler), hexuronic acid (Neuberg-Saneyoshi) and cholesterol (Liebermann-Burchard) were not detected. Osaki-Turumi test (the direct as well as the indirect) was also negative. Sulfur (Lassaigne) was absent. Nitrogen (Lassaigne) and phosphorus (ammonium molybdate test) were present.

4. Quantitative properties.

Due to shortage of material quantitative analysis could be accom-

plished only on some of the final products, whose results are embodied in Table IV.

TABLE IV.

Composition of A-S.S.'s and B-S.S. I.

Substance / Analysis	A-S.S. I	A-S.S. II	B-S.S. I
	In per cent		
Galactose			
Indole r.	13.0	16.2	10.0
α-Naphthol r. I	12.4	16.2	10.4
Mean	12.7	16.2	10.2
N	2.88	3.64	3.39
NH_2-N	1.03	1.21	1.59
P	4.27	5.60	3.35
Sphingosine	22.9		
Insoluble fatty acid	29.8		
Ash	4.95	4.92	4.70
	In equivalents per equivalent weight		
Galactose	1.0	1.0	1.0
N	2.9	2.9	4.3
NH_2-N	1.0	0.96	2.0
P	2.0	2.0	2.1
Sphingosine	1.1		
Insoluble fatty acid	1.2*		

* Calculated out with the molecular weight of the fatty acid of 364.

Analytical methods.

The sugar (galactose): By the ordinary colorimetric method it is not estimated so accurately in lipoids as in polysaccharides. The figures obtained on psychosin sulfate and cerebron, which contain 35.3 and 21.7% galactose respectively, were the following,

Substance	Percentages obtained by the methods of			
	Orcinol	Indole	Diphenylamine (I)	α-Naphthol (I)
Psychosin sulfate	29.0	35.0	20.0	33.0
Cerebron	19.7	19.7	35.0	20.0

From the result the writer preferred the indole and α-naphthol (I) methods to the others and applied them in smaller scales (1/3 and 1/2) than the original.

Nitrogen. By micro Kjeldahl method.

Phosphorus. By Haneda, Sinokawa and Yasuoka's modification of Plimmer method.[3]

Amino nitrogen. By micro Van Slyke method.

The insoluble fatty acid and sphingosine in A-S.S. I. 13.43 mgm. of the substance were

heated with 3 cc. of 2 n. H_2SO_4 solution, as sealed in a tube, at 100°C for 3 hours. When cold, the deposit was collected in a weighed filtros with water (six 2 cc. portions of water were used to wash off sulfuric acid completely) and dried constant in a desiccator. After weighing, the content of the filtros (the total insoluble fatty acid plus sphingosine; 7.08 mgm.) was agitated with many 5 cc. portions (50 cc. in total) of 1% NaOH with suction and again washed with water thoroughly. Dried. The remainder in the filtros (sphingosine) amounted to 3.08 mgm. (this gave the positive Thudicum reaction). So 4.00 mgm. (=7.08-3.08 mgm.) was the insoluble fatty acid. The alkaline filtrate and the washings were combined, neutralized (with HCl) and evaporated under diminished pressure to about 5 cc. and 1% HCl added to pH 2.0. The appearing precipitates were transferred to a centrifuge tube and washed with water. When thus all HCl was eliminated, they were suspended in 3 cc. of water and titrated to pH 8.6 (to thymolblue) with 0.01 n. NaOH (all particles were dissolved). 1.10 cc. of the alkali were used. Therefore 364 $\left(=\frac{0.004\times 1000}{1.10\times 0.01}\right)$ was its N. E., which coincides with the corresponding values of lignoceric acid (368) and nervonic acid (366).

Neutral equivalents and molecular weights:—

The substances involved one primary amino radicle or two. Hence, formol titration was employed to determine their equivalent weights.

A-S.S. I. 10.96 mgm. of A-S.S. I were taken up in 4 cc. of redistilled water and 0.01 n. NaOH was added to pH 8.4 to cresolred and thymolblue (impregnated in strips of paper), then after addition of 1 cc. of 16% formaldehyde solution at the same pH, the alkali was further added to shift the pH back to 8.4. The blanc analysis was carried out without the substance. The difference between the consumed total quantities of alkali was 2.60 cc.

$$\text{N.E.}=\frac{0.01096\times 1000}{2.60\times 0.01}=422.$$

When assumed that the molecule possesses 3 acid rests, the molecular weight is computed at 1266 (corrected for the ash content: 1203). The content of phosphorus, whose 2 atoms at least are contained by one molecule of the substance, suggests the molecular weight of 1452 $\left(=\frac{31\times 2}{4.27}\times 100\right)$ (1380 on an ash-free basis).

A-S.S. II. Calculated out from phosphorus, its molecular weight amounts to 1110.

B-S.S. I. The molecular weight from phosphorus reaches 1740. The neutral equivalent was 384 (10.43 mgm. of the substance consumed 2.6 cc. in total of 0.01 n. NaOH). To obtain herefrom a molecular weight approaching the above the substance has to be a tetravalent acid ($394\times 4=1576$). But it is conjectured that B-S.S. I is still contaminated with no small amount of substances possessing nitrogen and acid rests (s. below).

Discussion.

Clear-cut separation of lipoids from a mixture is often extremely

troublesome because of the mutual effect on solubilities and one kind of lipoid is liable to disseminate among various fractions. Not only from such a ratiocination but also from the fact that their equivalent ratios of the ordinary sugar, nitrogen and phosphorus, components most probably participating in the group reaction, are similar, S.S.'s I and II from A erythrocytes are legitimately assumed to be essentially identical with each other. And moreover, II appears purer than I for the reasons: 1) more of psychosin (calculated from galactose) which is one of the determinant groups of group(ABO)-specific lipoids (cf. Masamune[4]), is present in II, and 2) II is superior to I concerning the sero-reactive potency.

S.S.'s from B erythrocytes differed regarding solubility and composition from those from A erythrocytes. But we know that the group-glycidamins or polysaccharides in one kind of tissue or excretion (mucosa of stomach and of small and large intestine,[5] gastric juice,[6] urine,[7] gastric cancer[8]) do not diverge from one blood group to another in the mentioned respects and that the same nearly applies to the group lipoids in liver. Consequently, impureness of B-S.S.'s is rather assumable than unlikeness between the group lipoids from B and A erythrocytes. Since B-S.S.'s are more soluble in organic solvents than A-S.S.'s, they probably contain ordinary lipoids as impurities. Their further purification was not achieved because of lack of material.

In short, the writer inclines to believe A-S.S. II most approximately represents the composition common to the group active factors of lipoid nature in red cells irrespective of the blood group. Nitrogen in that fraction exceeded the amount equivalent to sphingosine, supporting the view of Masamune[4] on the determinant structures of group-specific substances, and, of its three atoms one was found in the form of the primary amino radicle. The equivalent ratio of phosphorus to nitrogen was 2:3. Phosphate rests might play the rôle of ionic attraction towards the agglutinin as probably does the sulfate rest in group carbohydrates and glycidamins. The writer thinks also that both the A- and B-group erythrocyte-lipoids possess the solubility of A-S.S. II.

Co-adsorbents from cells of different groups promoted the action of A- and B-S.S.'s similarly, so that they appeared to contain an identical substance. Their resemblance to S.S.'s in composition and solubility so far as tested is of significance.

Summary.

1. Strong group-specific lipoid-fractions were obtained from red blood cells.

2. From the physical and chemical properties of those fractions and for reasons by analogy the writer considered: a) the group lipoids from

the present source contain galactose, sphingosine, nitrogen and phosphorus in the equivalent proportions of 1 : 1 : 3 : 2 in common, and the content of the sugar is nearly 16%; b) these active factors possess a solubility atypical of lipoids, namely, they give a complete solution in hot water and are difficultly soluble in such usual organic solvents as alcohol, ether, petroleum ether and acetone.

3. A "co-adsorbent" is present in erythrocytes. It resembles the group specific lipoids in composition (only qualitatively examined) as well as in solubility and promotes the sero-reaction of these.

Through the Grant Committee for Scientific Researches a grant was given from the Education Department towards the expenses of this investigation, which is gratefully acknowledged. H. Masamune.

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The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

Studies on the Antibiotic Substances from Actinomyces.

(6th Report)

On the Properties of Several Kinds of Streptothricin-like Substances.

By

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(Received for publication, December 26, 1949)

1) Five kinds of basic antibiotic substances, resembling streptothricin in nature, were obtained in forms of hydrochlorid, crystalline helianthate and reineckate and the identification of these substances was performed.

2) All of these substances had lower potencies than streptomycin in a dilution test against E. coli and were arranged in the order of increasing toxicities as follows:

36, Streptomycin, 120, 24, 39 and 259.

The substance No. 36 was able to be injected into mice in an amount of 20 mg without showing any toxic signs.

3) The melting or decomposition points of helianthates or reineckates of 5 substances were specific for each substance and found in the vicinities of those of streptomycin and streptothricin. Maltol and Sakaguchi's reactions were positive in streptomycin alone, while glucosamine and other sugar reactions were positive in both streptomycin and streptothricin-like 5 substances.

4) A strain of E. coli, which was made resistant to the hydrochlorides of 3 substances (36, 259 and 120 respectively), were resistant to each of 5 substances and somewhat less resistant to streptomycin too, while the same strain of E. coli, which was made resistant to streptomycin, was not resistant to each of 5 substances.

In these respects it seems very probale, that each of 5 substances was quite different from streptomycin and somehwat different from streptothricin and from one another, though they resembled in many chemical and biological properties.

The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

The Blood Group Substance in Red Blood Cells.

Second Report.

Is any Group Polysaccharide Existent in Erythrocytes?*

By

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(Received for publication, December 25, 1948)

To isolate the group-specific substance, Hallauer[1] extracted with dilute alcohol the erythrocytes, which had been dried and treated with boiling alcohol, and in a few cases stromata. The sero-reactive products after eliminating water-insoluble contaminants did not dissolve in alcohol, ether, acetone and cold benzene but did dissolve in water. They contained N, P and S and their acid hydrolysates gave the positive Molisch, phloroglucinol and Bial reactions for sugar and reduced Fehling solution. On these findings the author believed the presence of a carbohydrate-complex as well as phosphatide- or cerebroside-like substances in his preparations. Later on, Kossjakow[2] put forward the polysaccharide nature of his substances, which had been obtained in a way like Hallauer's, starting with extraction of fresh erythrocytes immediately with 16% alcohol. His claim was based on properties of the substances: (1) solubleness in water and insolubleness in alcohol, ether, acetone and chloroform (he regarded this solubility as an evidence against the lipoid nature of the substances); (2) non-precipitability by trichloracetic and sulfosalicylic acid and negative biuret and xanthoprotein tests; (3) positive Molisch reaction and reduction of Fehling solution after hydrolysis; (4) non-dialysability.

Nevertheless, positive colour reactions for sugar and the reducing force appearing on hydrolysis can not be accepted as proofs of polysaccharide, because cerebrosides which contain only one single sugar rest in the molecule, show these properties and furthermore ordinary lipoids will become soluble in water by an additional component holding many OH-groups as inositol or by attachment of a sufficient number of positive and negative polar groups forming an inner salt. Such examples are the group-specific lipoids from red blood cells[3] and cancer tissue[4] and possibly also Bang

* 124th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates."

and Forssman's[5] and Takaki's[6] hemolysinogen, Landsteiner and Levene's heterogenic antigen[7] and Fischer's syphilisantigen.[8]

Now the presence in erythrocytes of the group substance belonging to a kind of lipoid in wide sense has been established, it is conceivable that Kossjakow could extract it with very dilute alcohol owing to its atypical solubility. To investigate whether or not a carbohydrate, that is group-specific, exists in erythrocytes, the writer shook the erythrocytes, that had been treated with alcohol and ether, with dilute alcohol in one case and with water in the other. The substances essentially soluble in water were followed up in the extracts and finally fractioned by the aid of glacial acetic acid, an expedient agent to purify group-specific carbohydrates[9].

However, not only the starting materials and the intermediate fractions but the final products were all unable to inhibit isoagglutination of erythrocytes. And moreover, the final products did not appear to be a polysaccharide, because, on one hand, they were soluble in benzene and chloroform besides water and contained psychosin (Thudicum test), resembling the group lipoids in the first report[3], and, on the other, they did not give Osaki-Turumi test for hexosamine which the specific carbohydrates possess in common.

Most probably the substances of Hallauer and Kossjakow were a lipoid and it looks to the writer that erythrocytes have no group polysaccharide nor any inactive polysaccharide.

Experimental.

Trials to Separate the Hypothetical Group Carbohydrate.

Experiment 1.

500 cc. of blood from individuals belonging to Group A were centrifuged and the erythrocytes were completely separated from the serum by washing with physiological saline. The porridge of erythrocytes (160 cc.) was kept in 5 volumes of a mixture of 95% alcohol and ether (1:1 by volume) for 1 week, shaking occasionally, and dried in a vacuum desiccator (H_2SO_4) to dryness. The residue (50 gm.) was boiled with 300 cc. of 95% alcohol under reflux twice (for 10 and 8 hours) and further exhausted with ether in a Soxhlet apparatus until no colouring substance came out. The obtained material (no anti-agglutinative potency observed at 1:100 dilution) amounting to 45 gm. was used for separating the alleged carbohydrate.

It was extracted twice by 4 days' shaking with 3.4 *l.* of 16% alcohol (cf. Hallauer[1] and Kossjakow[2]). The filtrates (The undissolved residue (Fr. Pr.) was employed for *Exp. 1* in the forthcoming report, "Is any group

protein present in erythrocytes ? ") were condensed together under reduced pressure to about 30 cc. and the appearing precipitates centrifuged off. To the yellowish supernatant liquid were added 5 volumes of acetone and the flocculent precipitates centrifuged, washed with acetone (three 2 cc. portions) and dried. 45 mgm. of yellowish powder were yielded. This gave the positive Molisch test, but was free from biuret-giving substances. The inhibitory potency to the isohemoagglutination was not recognized at all even at a dilution of 1 : 100.

To remove water-insoluble substances perfectly, the above product (40 mgm.) was subjected five times to redissolution in 5 cc. of water and reprecipitation by the aid of 25 cc. of acetone (each time, the insoluble part was rejected and the precipitates washed with acetone and dried), and at the end precipitated from 3 cc. of water (The insoluble part did not reach beyond a trace) with 20 cc. of absolute alcohol and washed with absolute alcohol and dried. The product was further divided into two portions, the soluble and insoluble in glacial acetic acid (98%), by employing 5 cc. of this solvent. The centrifuged insoluble part was washed thoroughly with absolute alcohol and dried—non-coloured powder, 15 mgm. The supernatant liquid was precipitated with ether (30 cc.), washed with ether and dried—yellowish, somewhat sticky powder, 2 mgm. These fractions were both water-soluble, but exhibited no anti-isohemoagglutinative activity at a dilution of 1 : 100. They gave Thudicum's reaction in addition to Molisch's. Their other properties will be seen below.

Experiment 2.

From the residue R of A erythrocytes in the foregoing report (see the section concerning the preliminary fractionation), 120 gm. were taken to shake with 600 cc. of water (3 cc. of 20% thymol solution in alcohol were added for preservation) for 5 days. The centrifuged supernatant liquid (red-brown and transparent) was evaporated under reduced pressure to about 7 cc. and the occurring precipitates (small in amount) centrifuged off. To the supernatant fluid, which was biuret-positive, 1 gm. of trichloracetic acid was added. The precipitates were discarded and again the mother liquid precipitated with 30 cc. of 98% alcohol. The precipitates here were washed with 30 cc. of 95% alcohol three times and dried in vacuo. The yellowish powder obtained (110 mgm.) was water-soluble. It was Molisch-positive and biuret-negative but not inhibitory (tested at 1 : 100 dilution) to the isoagglutination of A erythrocytes.

Treatment with glacial acetic acid was made as follows. The substance was at first kneaded with 2 drops of water and then agitated with 20 cc. of glacial acetic acid. After further addition of 0.2 gm. anhydrous

sodium acetate, the mixture was centrifuged and the centrifugate washed many times with absolute alcohol and dried—white powder, 30 mgm. To the supernatant liquid were added three volumes of ether and the precipitates separated, washed and dried similar to above—yellowish sticky powder—10 mgm. These products resembled the corresponding products in *Exp. 1* regarding solubility as well as sequels of the chemical and serological tests.

Properties of the Final Products.

The solubilities of the glacial acetic acid-insoluble fractions are illustrated in Table I and the results of tests of all the final products in Table II.

The glacial acetic acid-insoluble fraction in *Exp. 1* contained 3.5% ordinary sugar as galactose (by the indole method) and 3.0% phosphorus and that in *Exp. 2* 10% ordinary sugar as galactose (by the same method as above), 1.8% nitrogen, 12.3% phosphorus and 37.4% ash.

The expenses of this work were defrayed by a grant given from the Education Department through the Grant Committee for Scientific Researches.

H. Masamune.

Table I.

Solubility of the Glacial Acetic Acid-insoluble Fractions.

(Ins. fr. =glacial acetic acid-insoluble fraction. −insoluble, +partly sol., ++mostly sol., +++completely sol.)

Solvent	Fraction	Ins. fr. in *Exp. 1*	Ins. fr. in *Exp. 2*
Water	cold	++	+++
	hot	+++	+++
Gl. acetic acid	cold	−	−
	hot	−	−
Alcohol	cold	−	−
	hot	−	−
Acetone	cold	−	−
Ether	cold	−	−
Chloroform	cold	++	+
Benzene	cold	+++	+++
	hot	+++	+++
Tetralin	cold	+	+++
	60°C	++	+++
Petr.-ether	cold	−	−

TABLE II.

Results of Chemical Tests.

("Sol." and "Insol." signify the products soluble and insoluble respectively in glacial acetic acid. − negative, ± faintly positive, + plainly positive, ++ strongly positive.)

Test \ Fraction	*Exp. 1*		*Exp. 2*	
	Sol.	Insol.	Sol.	Insol.
Biuret	−	−	−	−
Osaki-Turumi				
Direct r.	−	−	−	−
Indirect r.	−	−	−	−
Molisch	++	+	++	++
Thudicum	+	+	±	+
Phosphorus	+	+	+	+

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Studies on Skin Pigment.

8th Report: Naevus fusco-caeruleus ophthalmomaxillaris OTA.

Preliminary Report.

By

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(Received for publication, December 27, 1949)

In 1938, Prof. Ota of Tokyo University first reported a variety of pigmented nevi which has since been known as naevus fusco-cæruleus ophthalmomaxillaris Ota or shortly nævus Ota in this country.

This disease is characterized by its peculiar blue-brown color reminiscent of brownish color of ephelides plus blue of blue nevi. Another characteristics is its location. It frequents in a patch mostly unilateral on the face, especially on upper and lower eyelids, zygomatic and maxillar region corresponding to the area of fifth brain nerve. Moreover, pigment spots are often observed on the conjunctiva of the affected side.

This disease is not so rare in Japan. The auther have observed 97 cases in the dermatological clinic of Tohoku University. Among these, 44 were located on the area of nervus ophthalmicus, 19 on nervus maxillaris and 29 on both two nerves. Melanosis bulbi was noted in 64% cases.

In the histological study, no "nevus cells" are found. Pigmented cells are located in the corium in the range from papillary layer down to the middle layer of the dermis, sometimes even in the depth of sweat and sebaceous glands. Their form, size and arrangement are irregular. Some take fusiform, stellate or string-like form, just like as one of blue nevus or mongolian spot. Their dopa reaction is weakly positive. So I incline to believe that they are of mesodermal origin and are possessed of melanogenic activities. And from the characteristic arrangement of this nevus, it is most probable that there exists certain relationship between neurohormonal reaction and pigment production as was suggested by Ito in the study of nevus cells.

The details will be published in a later issue of this Journal.

The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

On Proteins and Amino Acids I.

The Blood Group Substance in Red Blood Cells.

Third Report.

Is any Group Protein Present in Erythrocytes ?

By

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(Received for publication, December 25, 1948)

In 1926, Brahn and Schiff[1] said that human erythrocytes possess a group-specific protein-agglutinogen, which reacts with normal sera but not with immune sera and is water-soluble, non-precipitable by sulfosalicylic acid and dialysable. According to the authors, not only the residue of erythrocytes exhausted with alcohol and ether but also its watery extract exhibits the mentioned activity. But, as pointed out in the second report, the writer could not prove the anti-isohemoagglutinativeness of a fraction of red cells like that Brahn and Schiff examined and its water-soluble part.

Recently Beppu[2] again took up the problem and insisted that from stromata an active protein-fraction is obtainable by the following procedure: (1) erythrocytes are washed with physiological saline; (2) 50 gm. of the erythrocyte porridge are shaken with 5 volumes of ether and after separating off ether, 10 volumes of water added and the mixture stood in an ice chest overnight; (3) the precipitates are washed with distilled water and the gray stromata obtained are taken up in 20 cc. of 0.1% NaOH, removed from the insoluble part, neutralized with 0.1 n. HCl and mixed with an equal volume of the saturated ammonium sulfate solution; (4) the brown precipitates are collected on a filter, suspended in 50 cc. of distilled water and dialyzed against running water for two days; (5) the centrifugate is washed with water and its suspension in 20 cc. of physiological saline is kept in a thermostat at 37°C, with occasional shaking, for one day. A half-solution thus obtained was used by the auther as such for adsorption assay and the centrifuged supernatant liquid for precipitin assay. It is described that this half-solution showed protein reactions but not that of Molisch and was inactivated by trypsin.

Whether or not an anti-isohemoagglutinative non-glycidamin protein

is present is a very important matter, because a configuration, which is constructed of either hexosamine or sphingosine and either galactose or galacturonic acid, was hypothesized by Masamune[3] as one of the determinant groups of ABO antigens. Such a structure can not occur by linkage of amino acids among themselves. In order to dissolve the problem, the writer made two attempts—one, fractionation of a protein-mixture of erythrocytes after exhaution with alcohol, ether and dilute alcohol (cf. the foregoing report[4]), to see if any protein is separable whose anti-agglutinative potency is too low to be demonstrated in the mixture (*Exps. 1* and *2*) and, the other, fractionation of a half-solution of stroma proteins prepared by the method of Beppu (*Exp. 3*). In the former case, none of the obtained fractions gave the seroreaction even at 1:100 dilution. In the latter case, the half-solution actually inhibited the isoagglutination of erythrocytes, but the active factor passed into benzene on boiling the centrifugate of the half-solution, which contained most of it, with this solvent, and further gave the positive chemical tests that the group lipoid informed of in the first report[5] did.

Experimental.

Experiment 1.

This experiment was started with A erythrocytes which had been exhausted with alcohol (cold and hot), ether and 16% alcohol in sequence (the material obtained in *Exp. 1* in the second report[4]). It was divided first into fractions soluble and insoluble in a mixture of acetic acid and alcohol. With this aim, 1 gm. of the substance was kneaded in a mortar with 20 cc. of glacial acetic acid to a porridge, diluted with an equal volume of water and 30 cc. of 95% alcohol added. To the centrifuged supernatant liquid (60 cc.) was added double the volume of ether and the precipitates washed with changes (four) of alcohol and dried in a desiccator—Fr. Etn (yellow-brown powder, 120 mgm.)—and the mother liquid distilled under reduced pressure to dryness—Fr. Ets (red-brown powder, 150 mgm.). The centrifuged residue after treatment with acetic acid and alcohol (above) was washed with alcohol repeatedly (Five 20 cc. portions were used; the final washing had a pH of 5.0) and dried in a desiccator—700 mgm. It was then stirred up with 5 cc. of water and 1/3 n. Na_2CO_3 solution added to pH 9.6 (About 2 cc. of the carbonate solution were required). The mixture was shaken on a machine for 24 hours and the separated extract (yellowish opalescent) was precipitated by addition of 5% acetic acid till pH 5.0. The precipitates were centrifuged, washed well with alcohol and dried—Crude Fr. Alks-acidn I. To this was again applied a similar procedure of extraction and precipitation and 15 mgm. of orange powder were yielded

—Fr. Alks-acidn I. From the mother liquid of the crude fraction (Crude Fr. Alks-acidn I) were again obtained precipitates on addition of additional acetic acid (1 cc. of 1% acetic acid; pH was lowered to 4.8)—Fr. Alks-acidn II (10 mgm., brownish powder). Not only the starting material but also Frs. Etn, Ets, and Alks-acidn's I and II were not seroactive at a dilution of 1:100.

Experiment 2.

Of the same batch of the material used in *Exp. 1*, 7 gm. were extracted with 40 cc. of 0.1% NaOH by shaking on a machine for 2 days (0.2 cc. of 5% alcoholic thymol solution was added to prevent putrefaction). The red-brown filtrate (pH, 7.4) was acidified to pH 5.0 with 0.1 n. HCl, whereupon maximum precipitation occurred. Centrifuged. The centrifugate was dissolved in 15 cc. of water and again precipitated with 3 volumes of alcohol. The precipitates were washed with alcohol and dried. The product obtained (red-brown powder, 90 mgm.) showed no anti-isohemoagglutinative activity at a dilution of 1:100.

Experiment 3.

A half-solution of stroma proteins (Beppu) was prepared from B erythrocytes by following the original method. It gave plain Molisch and distinct biuret reaction and inhibited the isoagglutination of B erythrocytes up to 4 times dilution. A portion of it was centrifuged to measure the serological activities of the suspended particles and the solution separately. The suspension of the centrifugate at the original dilution in physiological saline showed the activity as strong as the half-solution itself, whereas the supernatant fluid (Molisch ±, biuret +) was no more inhibitory to the agglutination when diluted twice, in other words, the serological potency was found to reside mostly in the undissolved part. Next, the suspension of the centrifugate was sealed and heated in a boiling water-bath for 30 minutes, but its activity was not reduced, indicating the active factor heat-stable. After these preliminary observations, the centrifugate of the half-solution was fractioned as follows.

10 cc. of the half-solution were centrifuged, and the centrifugate was extracted with 40 cc. of ether, and then with 40 cc. of a mixture consisting of equal volumes of ether and benzene. After drying in vacuo, it was agitated with 40 cc. of benzene at 70–75°C three times and subsequently with 80 cc. of the ether-benzene mixture in the cold. Dried in vacuo, the residue amounted to 40 mgm. 4 mgm. of it were swelled in 1 cc. of physiological saline by means of alkali (our usual technique adapted) for the serological test. (The substance was diluted thereby to a similar degree

as in the half-solution.) The suspension itself was anti-agglutinative, though not when the dilution was doubled. Therefore, the remaining part (36 mgm.) was again treated with benzene twice, by boiling each time for 2 hours under reflux. The substance was reduced to 26 mgm. It gave the biuret reaction distinctly but Molisch's scarcely and there was no indication of inhibiting the isoagglutination of B erythrocytes at its dilution as in the original half-solution. On the other hand, the evaporated rest (8 mgms.) of the benzene and ether extracts (distilled together at a low temperature) was found active at a dilution of $1:2\times10^3$ which corresponded nearly to double its dilution in the half-solution and free from biuret-giving substances and gave the positive Thudicum (for psychosin) and Molisch (for sugar) tests besides Liebermann-Burchard's (for cholesterol).

On these results it is most plausibly assumed that the active factor in Beppu's half-solution was the group lipoid adsorbing on inactive proteins.

Immediate extraction of the half-solution with cold benzene and ether was almost ineffective for separation of the specific lipoid.

Conclusion.

In erythrocytes no group substance of protein nature is considered as existent.

Through the Grant Committee for Scientific Researches was furnished a grant from the Education Department for defraying the costs of this work, which is gratefully acknowledged. H. Masamune.

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On the Paraganglia in the Ganglion of the Vagus Nerve.

By

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Recently the Japanese Surgical Society has undertaken a big problem which is the resection of the carotid paraganglion as a surgical treatment for bronchial asthma. This carotid paraganglion belongs, as do as the cardiac paraganglia discovered by Penitschka[1] and Seto[2] in 1931–35, to a sort of gland of internal secretion which from various points of view stands in opposition to the medullary substance of the suprarenal gland.

A comparison of these two groups of ductless glands set up in opposition each other reveals that though they belong to the nervous accessory organs (Kohn,[3] Kose,[4] Watzka[5]) standing in intimate relationship with blood vessels and nerves, there are between them following the differences.

1. Embryologically: The carotid and cardiac paraganglia appear relatively late in foetal time, but they remain throughout life. On the contrary, the medullary substance of the suprarenal gland and other paraganglia of the same kind (aortic paraganglia of Zuckerkandl[6] etc.) appear early in foetal time, but soon after birth gradually recede and disappear leaving only slight microscopical evidence, one exception being the medullary substance of the suprarenal gland, which remains throughout life.

2. Innervation: The innervation of the carotid and cardiac paraganglia are supplied mostly through the parasympathetic nerves, especially by the vagus, while the medullary substance of the suprarenal gland is innervated through the sympathetics.

3. Cytologically and physiologically: The parenchyma of the sympathetic paraganglia consists of chromaffine cells. The extract of this parenchyma contains adrenalin which causes the rise of blood pressure. The parasympathetic paraganglia on the contrary have far fewer chromaffine cells, or at times these are even absent (Kose,[4] Watzka[5] etc.). And according to many investigators the extract of these causes a fall in blood pressure.

As pointed out above (embryologically, physiologically etc.) the carotid gland and the cardiac paraganglia both belong to the ductless glands standing in opposition to the medullary substance of the suprarenal gland. Then on the basis of above shown similarity, if the resection of the carotid paraganglion is effective in the treatment of bronchial asthma, the resection of both the carotid and cardiac paraganglia should be consequently much more effective. In addition, since the functions of these paraganglia belonging to the parasympathetics rely upon the aortic, carotid sinus and cardiac reflexes of Hering,[7] it follows that by the resection of the sensory nerves involved with this reflex fall of blood pressure, such as the aortic nerve (formerly termed N. depressor and partly distributed in the auricle) and the sinus nerve the bronchial asthma should be similarly relieved. If this is true it likeweise follows that simultaneous resection of both parasympathetic paraganglia and sensory nerves, each dependent upon the other, should be much more effective than resection of either one of them.

Seto,[2] one of our investigators, expressed the opinion in his report (1935) on the cardiac paraganglia, that the parasympathetic paraganglia would not only be situated at the two places described above, but distributed in other places along the course of the vagus nerve also. Muratori[8] in 1932 discovered such a paraganglion, in- and outside of the ganglion nodosum of the vagus nerve in birds. But now we can not oberlook the work of White,[9] who has found out recently by chance in his histological study of the carotid gland that there is a special new organ with great similarity to the carotid gland accompanied by the ganglion nodosum of the vagus nerve in the human. However, he had to refrain from publishing his decisive diagnosis as a paraganglion, because he did not study on its innervation with the silver impregnation method.

Recently we have studied the root part of the vagus nerve in the adult man by longitudinal frozen sections stained by Seto's modification of Bielschowsky's silver method. We were able to find very small groups of cells (paraganglia) having the structure like the carotid or cardiac paraganglia. They are generally of small size, scattered in the ganglion nodosum of the vagus, chiefly in the connective tissue between the bundles of nerve fibres. In comparison of size these are only about one fourth of the size of the cardiac paraganglia and about one half of the size of the carotid paraganglion.

In the microscopical examination of these paraganglia of the ganglion nodosum, as shown in the Fig. 1, the parenchymal cells are all clear, containing a round or oval nucleus, and grouped to form the lobules, which are invested by the interlobular connective tissue. The parenchyma is rich in blood vessels, especially in fine veins and capillaries, and is characterised by the peculiar distribution of nerve fibres. The extracapsular

nerves supplying these paraganglia consist of mostly relatively thick fibres belonging to the vagus nerve (parasympathetic) and partly very fine fibres (sympathetic type). These fibres penetrate the capsule and enter the parenchyma of the lobules. Under low power magnification the terminal fibres of the parasympathetic nerves (vagus) can be seen distributed throughout the parenchyma. These fibres vary considerably in thickness and appear to terminate in a dendritic pattern (Fig. 1). However under high

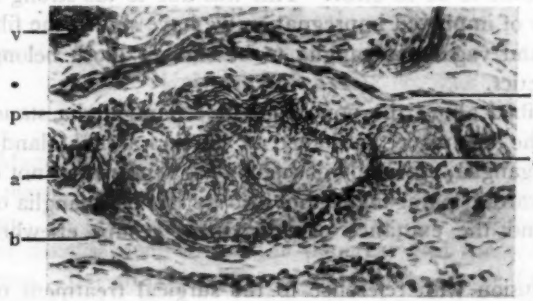

Fig. 1. Nonchromaffine paraganglion inside of the ganglion nodosum of the vagus nerve in the human. v. nerve bundle of vagus; b small artery; v small vein; p nerve plexus in the capsule; i interlobular nerve plexus; small veins and special nerve fibres found out so richly in the gland parenchyma. The *Seto*'s silver method, 350 times magnified, 1/3 diminished.

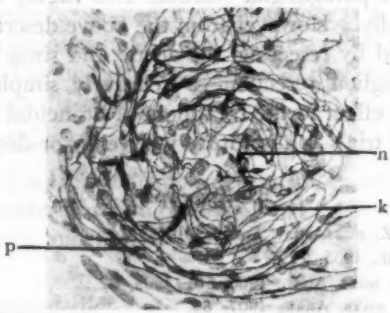

Fig. 2. The same paraganglion, showing the manner of nerve ending in an aggregation of gland cells. k nucleus of gland cells; p nerve plexus arround the cells aggregation; n terminal network of special nerve fibres in the cells aggregation, which indicate the great variety of thickness. The same staining, 900 times magnified, 1/2 diminished.

power magnification one can see that these end branches further divide and ramify forming a very complicated network of fine anastomoses which lie in close approximation to the parenchymal cells (Fig. 2). This ter-

minalreticulum is very similar to that found in the medullary substance of the suprarenal gland (Stöhr)[10]. However comparison of this terminal-reticulum of the parasympathetics (vagus) in these paraganglia with those of the vegetative (autonomic) nerves found in the walls of blood vessels and in smooth muscle tissue etc. reveals a marked difference in the fine structure. The very delicate and complicated terminal-network described in the cardiac paraganglia by Seto in earlier investigations (1933) has since been shown to be in error. This was due to too strong silver stain. Today by use of improved impregnation we have shown the fibres of both the cardiac and vagal paraganglia to be similar—both belonging to the parasympathetics.

As described above the nonchromaffine paraganglia standing in opposition to the medullary substance of the suprarenal gland—parasympathetic paraganglia, especially of vagus—are represented not only by the cardiac and carotid paraganglia, but also by the paraganglia of the vagus ganglion. And the existance of other such groups elsewhere may be supposed.

In conclusion with reference to the surgical treatment of bronchial asthma the resection of the carotid paraganglion can be done very easily. The simultaneous resection of the cardiac paraganglia and the paraganglia in the vagus ganglion with the above postulated effect would then offer a perfect cure for bronchial asthma. Unfortunately in practice it is impossible to resect these paraganglia (cardiac and vagus) because of their inaccessibility surgically. However from the above description this end may be partially realized by resecting the aortic and sinus nerves at the time of carotid paraganglion resection—a relatively simple procedure. The resultant beneficial effect in the treatment of bronchial asthma is here only postulated, clinical trials in the future will verify or disprove this theses.

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Studies on Blood Diastase.

The Sixth Report. Function of Blood Diastase.

By

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(Received for publication, February 11, 1949)

Since Magendie[1] discovered for the first time in 1840 the diastase in the blood, many investigators have concerned themselves with its origin and function. At present it is generally recognized that a large portion of the blood diastase comes from the pancreas and the rest from other various organs. But workers differ in opinion as to the function of the diastase in the blood. Carlson and Luckhardt[2] and later Moeckel and Rost[3] believe the blood diastase is functionless, being byproducts of organ metabolism which appear in the circulation on the way to destruction or elimination. According to my former experiments[4] the diastase pours out into the blood, like internal secretion, for the most part from the pancreas in proportion to its activity. Therefore it is presumed that the blood diastase is an indicator of the functional state of the pancreas. The present experiment has been undertaken to throw some light upon the function of the blood diastase.

Experimental Methods.

As in the case of my former experiments, rabbits were used after fasting for 24 hours. The determination of the blood diastase and sugar was done as in previous reports, and that of the blood glycogen by my own micro-method.[5]

Results of Experiments.

1. Glycogen, Sugar and Diastase in the Blood by Glycogen Injection.

As shown in Table I the glycogen content in the whole blood and plasma increases of course immediately after intravenous injection (10 cc. per kilo of 25% or 10% glycogen), but the blood sugar level reaches the maximum height slowly. As Bucher and Staub[6] reported, the data presented in these experiments show that a greater part of the injected glycogen stays in the plasma and disappears so slowly that it does not re-

turn to the previous level even in 5 hours.

As for the effect of intravenously injected glycogen upon the blood diastase, Sikinami etc.[7] contend at a great variance among them.

Table II demonstrates that the blood diastase level reaches the maximum in 4 hours. When 2 mgrm. per kilo of atropine sulphate is subcutaneously injected 15 minutes before injecting glycogen, the blood diastase increases all the same, although the effect is not so remarkable as in the above mentioned experiments. Therefore atropine sulphate does not prevent the rise of the blood diastase level so effectively as it does that of the blood sugar concentration. By the way, the intravenous injection of inulin, which is performed for a comparison with glycogen, does not raise the glycogen, sugar and diastase in the blood.

Because the liver glycogen level, as shown in Table III, does not rise after the injection of glycogen, the above noticed disappearance of glycogen from the blood circulation is not yet explained, it is assumed, by a hypothesis of Staub and Golandas[8] that the liver may be able not only to pour out glycogen into the blood as such, but also to receive it as such.

2. Glycogen, Sugar and Diastase in the Blood by Pilocarpine Injection.

TABLE I.

Intravenous Injection of Glycogen.

		Before injection	After injection of glycogen (Time in hours)					
			0,5	1	2	3	4	5
Control	Blood glycogen (mgrm.%)	71	63	50	72	68	67	73
	Plasma glycogen (mgrm.%)	36	31	28	40	23	26	36
	Blood sugar (mgrm.%)	113	102	93	83	87	85	87
25% Glycogen	Blood glycogen (mgrm.%)	38	765	694	428	387		
	Plasma glycogen (mgrm.%)	15	764	464	402	341		
	Blood sugar (mgrm.%)	113	127	167	209	160		
10% Glycogen	Blood glycogen (mgrm.%)	55	788	600	385	270	231	181
	Plasma glycogen (mgrm.%)	36	723	487	345	231	203	153
	Blood sugar (mgrm.%)	97	135	120	115	103	95	90

TABLE II.

Glycogen Injection after Atropine.

		Before injection	After injection of glycogen or inuline (Time in hours)					
			0,5	1	2	3	4	5
10% Glycogen	Blood glycogen (mgrm.%)	21	475	347	259	196	141	107
	Blood sugar (mgrm.%)	89	127	123	113	95	92	82
	Blood diastase (mgrm.%)	720	902	933	1024	1073	1098	1025
10% Glycogen after atropine	Blood glycogen (mgrm.%)	21	505	430	269	152	147	125
	Blood sugar (mgrm.%)	89	113	128	118	97	89	87
	Blood diastase (mgrm.%)	796	1065	1016	995	1063	1098	1067
10% Inulin	Blood glycogen (mgrm.%)	31	36	30	26	28	27	26
	Blood sugar (mgrm.%)	94	90	82	88	83	84	81
	Blood diastase (mgrm.%)	884	910	918	892	834	821	743

It is now generally accepted that pilocarpine raises the blood sugar and diastase level, but lowers the liver glycogen content.

The results presented in Table IV show that pilocarpine hydrochloride used in doses of 5 mgrm. per kilo raises the diastase, sugar and glycogen in the blood, mainly in the plasma.

TABLE III.

Liver Glycogen after Intravenous Injection of Glycogen or Inulin.

	Liver glycogen (%)					
	Before injection	After injection of glycogen or inuline (Time in hours)				
		1	2	3	4	5
Laparotomy	1,09	0,94	0,64	0,36	0,71	0,62
10% Glycogen	1,94	2,26	1,97	2,17	1,41	1,14
10% Inulin	1,15	1,30	1,45	1,11	0,90	0,72

TABLE IV.

Pilocarpine Injection.

		Before injection	After injection of pilocarpine (Time in hours)		
			1	2	3
Diastase (mgrm.%)	in the blood	723	1208	1106	1445
	in the plasma	532	1050	1072	1284
Sugar (mgrm.%)	in the blood	138	211	209	205
	in the plasma	131	201	188	188
Glycogen (mgrm.%)	in the blood	66	68	72	103
	in the plasma	30	54	42	79

Therefore it is naturally conceded that glycogen in the liver cells pours into the plasma by pilocarpine injection, and that then the glycogen passed to the plasma is digested by the increased diastase in the plasma.

DISCUSSION.

It is generally accepted that the intravenous injection of glycogen leads to a considerable hyperglycemia. Jongh and Laqueur[9] contend that the above mentioned hyperglycemia is able to remove many symptoms caused by hypoglycemia.

On one hand in normal animals Boehm and Hoffmann[10] denied a glycogenury after injecting glycogen, but Terashima[11] accepted so slight a glycogenury as to make the disappearance of glycogen from the blood circulation unascertainable. On the other hand in depancreatized dogs Iljin[12] proved the increased sugar in the urine after the intravenous injection of glycogen. Moreover Lombroso[13] contends that the urine contains not only the increased sugar, but also the increased glycogen in a case similar to Iljin's.

According to Bucher and Staub[6] a greater part of glycogen either injected in the vein or added to the blood in a test-tube stays in the plasma and 80% of them disappears within one hour.

The results obtained in these experiments lead to conclusions that the intravenously injected glycogen stays in the plasma and raises the blood diastase level so high through the humor-neural-stimulated function of the pancreas that the plasma glycogen can be digested into the sugar.

On one hand many workers have concluded that a larger part of the blood diastase exist in the plasma; for example Guelzow[14] reports 83% and my results obtained in these experiments are 74%.

On the other hand many investigators contend that a greater portion of the blood glycogen content is found in the blood corpuscles; for example Bucher and Staub[6] report 80%, Golandas[15] 66%, Kanda[16] 60%, Sikinami etc.[7] 40% and my results shown in Table IV are 54%.

These experiments with regard to the blood sugar content have proved that the blood corpuscles contain only 5% of the sugar in the whole blood, as many investigators contend with one accord.

Therefore it can be safely concluded that blood glycogen and diastase are able to exist together in the normal state, but that the diastase in the plasma can easily digest glycogen poured out into the plasma from the liver cells or other organs.

Summary.

From these experiments the following conclusion can be brought out: The blood diastase is usefull for the carbohydrate metabolism, assisting in the hydrolysis of glycogen which is poured out into the plasma from the liver cells as such.

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Studies on the Influences of Mapharsen on Fixed Neoarsphenamine Eruptions.

Preliminary Report.

By

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(Received for publication, December 27, 1949)

It has been pointed out that severe side reactions are not so common with mapharsen as with neoarsphenamine. But as to the specifity in allergic cutaneous reactions, the difference between these two drugs has not been thoroughly studied. In connection with this aspect the auther made following clinical and histological examinations on five cases of fixed neoarshenamine eruptions.

All these reacted to injections of neoarsphenamine with appearance of erythema and increase of pigmentation at the site of former eruptions, while the administration of mapharsen remained entirely without effect exept in one case who showed similar reactions not only to mapharsen but also to dichlorophenarsine oxide.

In harmony with the mentioned findings, histological examination revealed what follows: According to Naegeli and Quervain, sections from the site of fixed eruptions were soaked in 1% solution of neoarsphenamine, 0.6% solution of mapharsen and in physiological saline solution separately and skin pieces from normal persons as controls were also treated in a like manner. After 24 hours they were fixed and stained by the usual method. Histological damages in the form of degeneration of vascular endothelcells in dermal layer and vacuoles formation in epidermal cells were detected only in specimen that had been taken from the patients and soaked in neoarsphenamine solution.

In short, many cases of fixed neoarsphenamine eruptions exhibited strict specific reactivity to neoarsphenamine, and mapharsen, which is closely related in chemical structure, gave rise to no clinical and histological changes nearly in all cases.

The details will be accounted for in a later issue of this Journal.

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A Summarized Note on the Potency of Granular Leukocytes.

By

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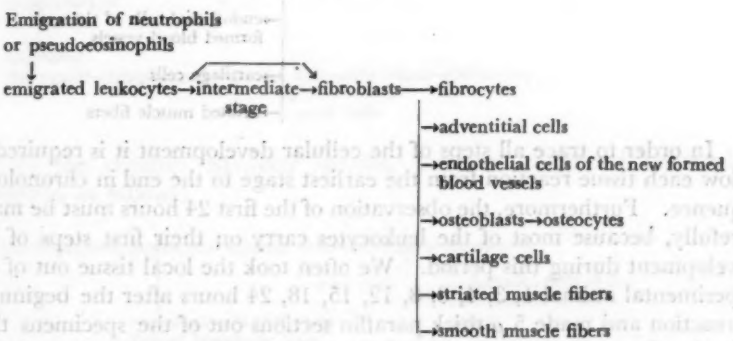
(Received for publication, February 17, 1949)

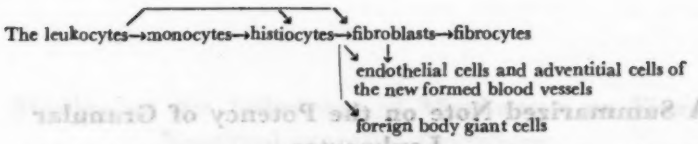
An Attempt will be made below to offer a perspective of the research which my co-workers and I have been carrying on since 1942. Its details may be reported in subsequent numbers of this journal.

On the first place there are good reasons for the conclusion that granulocyte, especially neutrophil or pseudoeosinophil, takes an exclusive part in the formation of granular and scar tissue in any inflammation or wound healing, as is seen in the following diagram:

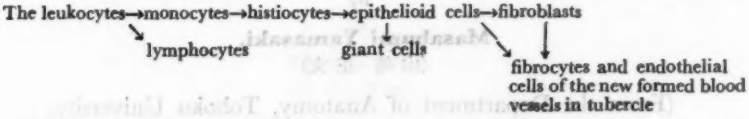


The behavior of the leukocyte in the intermediate stage changes under various conditions. Several cases will be mentioned below.

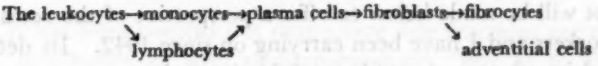
If the small piece of plant marrow, as an irritant body, is put into subcutaneous tissue of experimental animal, the leukocytes emigrate as is well known in large amounts from blood vessels shortly after the operation and travel toward the foreign body, but they behave in the subsequent stages as follows:


The leukocytes→monocytes→histiocytes→fibroblasts→fibrocytes
endothelial cells and adventitial cells of the new formed blood vessels
foreign body giant cells

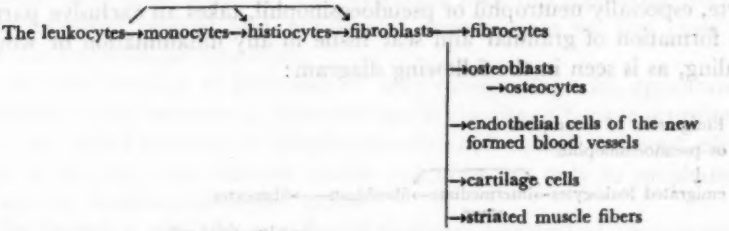
Histologic features of the intradermic reaction to turberculous factors


The leukocytes→monocytes→histiocytes→epithelioid cells→fibroblasts
lymphocytes
giant cells
fibrocytes and endothelial cells of the new formed blood vessels in tubercle

The process of Arthus phenomenon caused by egg albumin in the subcutaneous tissue has been traced as is shown below:



Repair of the fractured bone and the injured surrounding tissues:



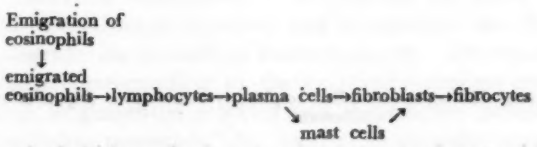
In order to trace all steps of the cellular development it is required to follow each tissue reaction from the earliest stage to the end in chronologic sequence. Furthermore, the observation of the first 24 hours must be made carefully, because most of the leukocytes carry on their first steps of the development during this period. We often took the local tissue out of the experimental animal 1, 3, 4, 6, 8, 12, 15, 18, 24 hours after the beginning of reaction and made 5 μ thick paraffin sections out of the specimens thus gained. For fixation was adopted Zenker-formalin solution, for staining, the method of Kull. Although the state of transformation of the leukocyte has not been actually followed by our eyes in the living cell, there is no longer any inconsistency in this process. In addition to this we have recently succeeded both in cultivating the purest leukocytes collected from the rabbit by the method of de Haan and in tracing their transformation into fibroblasts through the intermediate stages of monocyte or histiocyte.

Attension may be now directed to the tissue formation or maintenance under physiological conditions. It has been well known that the eosino-

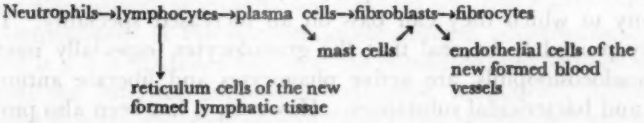
philic leukocytes appear to be of normal occurrence in lymphatic tissues in more or less a large amount. But it seems to be left a little uncertain at the present time what rôle the leukocytes are playing there. In the opinion offered by Maximow lymphocytes are able to transform into myeloic elements inclusive of eosinophils. Attension may be directed to the above mentioned result that in some tissue reactions lymphocytes were derived from neutrophils or pseudoeosinophils. The nature of lymphocyte as a distinct cell race considered to be of reticular origin may be, therefore, a subject of considerable speculation. Additionally in the lymph node of adult healthy animals all steps of transformation from eosinophil to lymphocyte were found out by us. At the same time there are no signs which may suggest cell multiplication in reticuloendothel, but only those of degeneration. Therefore, there is already a good reason for us to think as follows:

Eosinophils→lymphocytes→middle large and large→reticuloendothelial cells
lymphocytes (so-called lymphoblasts)

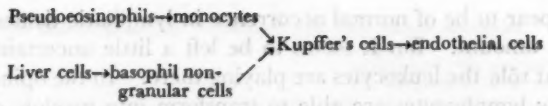
The diagram just shown above indicates that the process may be essentially in agreement with the result obtained from a number of nose polyps:



In the chronic maxillary sinuitis, however, emigrated neutrophils transform as follows:


Neutrophils→lymphocytes→plasma cells→fibroblasts→fibrocytes
mast cells
endothelial cells of the new formed blood vessels
reticulum cells of the new formed lymphatic tissue

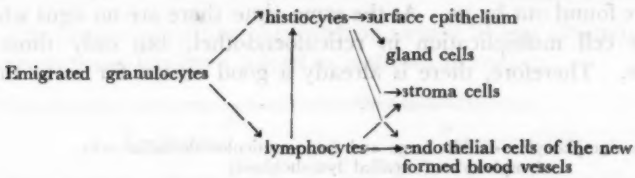
The mesenchymal origin of lymphocyte is undoubtedly proved to be real in early embryonic period, while the lymphocyte of adult organism as already stated arises from eosinophil as well as neutrophil or pseudoeosinophil under various conditions. On the other hand it was suggested at first in the lymphatic tissue that the reticuloendothel generally accepted as the rest of mesenchym should represent merely the final stage in the course of development of granulocyte. And this again is in agreement with our finding in rabbit liver:



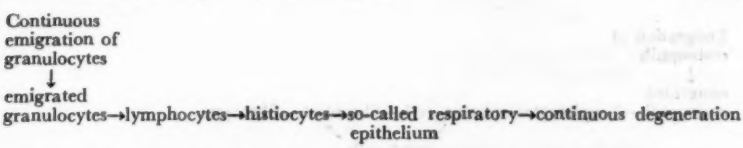
This process is stimulated by intravenous injection of typhoid vaccine.

The study on reticuloendothel of bone marrow is now in progress, naturally with reference to hematopoiesis and some favorable results have been already obtained.

The regeneration of menstrual uterus mucosa will be informed as one of the most marked cases of physiological regeneration:



The finding in the lung of a healthy rabbit may again suggest that the maintenance of the structural consistency in various tissues should chiefly depend on the potency of granulocytes:



Although we could not understand clearly what the basophilic leukocyte is, we could become conversant with the behavior of two other types of granulocytes. It must be now emphasized that these granulocytes, neutrophil or pseudoeosinophil and eosinophil, are not end cells but that they have progeny to which they can pass on an increased speciality. It is undoubtedly proved to be real that the granulocytes, especially neutrophils or pseudoeosinophils, are active phagocytes and liberate antitoxic, proteolytic and bactericidal substances. However, it has been also proved by us that the granulocytes take an exclusive part in the tissue formation under pathological as well as physiological conditions and it seems rather to be their original function.

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Delayed Conditioned Reflex in Man and Brain Waves.

By

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(Received for publication, February 26, 1949)

Introduction.

In Pavlov's theory[1] of conditioned reflexes, it is assumed that excitation and inhibition are two fundamental nervous processes in the cortex which control conditioned response of peripheral organs. But the existence of these processes was inferred only indirectly from the findings at peripheral organs, such as salivary glands or skeletal muscles.

Recently Motokawa,[2][3] investigated the brain waves in conditioned reflexes established in man, and interpreted the changes in electroencephalograms on the basis of Pavlov's theory. He reported that the potential pattern corresponding to the excitatory process consisted of reduced α-waves, augumented β-waves and "excitation potentials." With a term "excitation potentials" he referred to irregular waves of large amplitude with frequencies 3 to 5 per second, and he considered this sort of waves as an indication of strong excitation in the cortex. The "excitation potentials" disappeared and a train of well developed α-waves appeared in their place when the conditioned stimulus was repeatedly applied without accompaniment of the unconditioned stimulus (experimental extinction). Since it is a well known fact that the procedure of experimental extinction causes the internal inhibition (extinctive inhibition), Motokawa concluded that the disappearance of "excitation potentials" and development of α-waves are direct evidence of the existence of inhibition in the cortex.

The extinctive inhibition studied by Motokawa is a familiar example of internal inhibition. Another example of internal inhibition is the one found in delayed conditioned reflexes; when the interval between the beginning of conditioned stimulus and the administration of the unconditioned stimulus is large, the conditioned response does not take place until an appreciable time after the onset of the conditioned stimulus. Pavlov designated such a reflex as a "delayed conditioned reflex." As to the

mechanism of the delay of the conditioned response he put forwards the theory that a sort of internal inhibition is brought about by the conditioned stimulus and retards the appearance of the response.

The present investigation was undertaken to study patterns of electroencephalograms during delayed conditioned reflexes.

Experimental.

Method.

Brain potentials were picked up by a monopolar leading with a different lead on the vertex and with an indifferent one on the ear lobe. The potentials were amplified through a four stage resistance-capacity coupled amplifier and recorded by means of electromagnetic oscillograph (Yokogawa).

In a dark, quiet room the subject sat in a chair, with his eyes opened. The experimentor carried out the recording of the brain potentials and all the procedure of experiment in an adjoining room. As a conditioned stimulus a metronome was used which beated 116 times per minute. At the 20th second from the beginning of the conditioned stimulus, an electric lamp was switched on and switched off after five seconds. The metronome was kept beating for about 35 seconds. The electric light served as an unconditioned stimulus in the present experiment.

Results.

I. Changes of the Brain Potentials in the Course of Establishment of the Conditioned Reflex.

In the majority of experiment the subject was given the combined stimuli, the metronome and the electric light (procedure of reinforcement), but occasionally the former alone was delivered in order to test the effect of the conditioned stimulus. The paired stimuli were given five to ten times a day, and up to the end of the present experiment the total number of reinforcement amounted to 260.

As the conditioning proceeded, the electrical responses of the cortex to the paired stimuli changed in a characteristic manner. The diagrams in Fig. 1 show the typical potential patterns obtained at successive stage in the establishing course of the conditioned reflex. At the earliest stage slow irregular waves of large amplitude appeared at the onset of the conditioned stimulus and continued until the beginning of the unconditioned stimulus (pattern (a)). This kind of waves coincides with the "excitation potentials" described by Motokawa. As the conditioning was further strengthened, the "excitation potentials" became less continous and trains of α-waves of small amplitude could be seen between the groups of

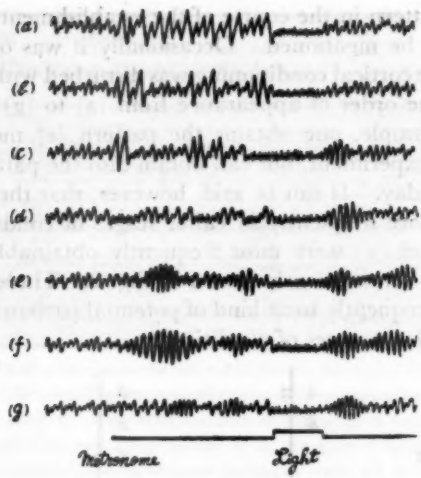

Fig. 1. Schematical diagrams showing the potential patterns obtained at the successive stages in the course of the establishment of the conditioned reflex. As the conditioning proceeded the pattern changed from (a) to (g).

'excitation potentials" (pattern (b)). In the following stage the "excitation potentials" were diminished in amplitude and β-waves were often observed in their place (pattern (c)). In addition to this fact it is worth noticing that the α-waves were momentarily developed after the end of the unconditioned stimulus. This phenomenon was interpreted by Motokawa as a post-excitatory induction which was caused by the preceding light stimulus. The pattern (d) is characterized by an alternative appearance of α- and β-waves during the first 20 seconds from the beginning of the metronome. No "excitation potentials" could be seen in this stage. Soon thereafter, the conditioning of the brain reached a stage in which the conditioned stimulus elicited no excitatory potential changes such as β-waves or "excitation potentials," but α-waves of increased amplitude (pattern (e)). In the subsequent stage such an effect of the conditioned stimulus was so marked that a regular train of well developed α-waves prevailed in the oscillogram during the first 20 seconds after the start of the metronome beats (pattern (f)). The post-excitatory α-rebound stated above became more pronounced as the conditioning proceeded. However, it is to be noted that an excess of conditioning could weaken the cortical response; as shown in the pattern (g) the potential changes due to the conditioned stimulus as well as the post-excitatory α-rebound were less marked in the final stages of the experiment than in the preceding stages.

The above statement refers only to the general principle of the changes

of the potential pattern in the course of the establishment. In this respect some details must be mentioned. Occasionally it was observed that the development of the cortical conditioning was disturbed without any external cause, and that the order of appearance from (a) to (g) was temporarily reversed. For example, one obtains the pattern (e) most frequently in some stage of the experiment, but can obtain also the pattern (b) or (c) on one and the same day. It can be said, however, that the patterns (a), (b) and (c) appear more frequently in ealier stages of conditioning, whereas the pattern (f) and (g) were most frequently obtainable in later stages. This circumstance can be clearly shown in Fig. 2. Three diagrams in this figure show how frequently each kind of potential pattern appears in ealier, intermediate and later stages of conditioning.

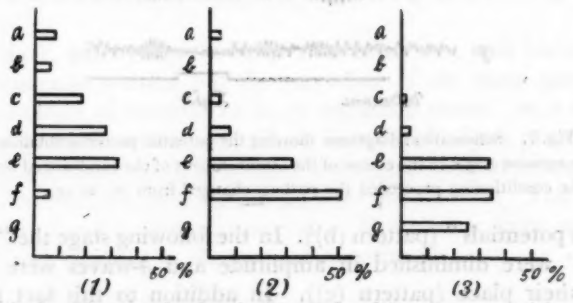

Fig. 2. Diagrams showing the frequencies of the appearance of the patterns in Fig. 1. The earlier (diagram (1)), intermediate (diagram (2)), and later stage (diagram (3)) of the establishing course contain the first sixty, the next one hundred and the last one hundred reinforcements respectively. In each diagram, the frequencies of the appearance of respective patterns are expressed in per cent of the total number of oscillograms taken in the respective stages.

On the basis of Pavlov's theory it seems likely that the pattern (f) corresponds to the completely conditioned state of the brain. The augumentation of α-waves in this pattern must be attributed to the internal inhibition in the delayed conditioned reflex (inhibition of delay). As the inhibition suppresses the excitation, no excitatory waves appear so long as the inhibition prevails. The excitatory waves are induced only during the administration of the unconditioned stimulus. In this stage the conditioning of the brain is so complete that, in spite of the absence of the unconditioned stimulus, conditioned excitatory waves can spontaneously appear about 20 seconds after the onset of the conditioned stimulus. An oscillogram of such a case is shown in Fig. 3 c. In about a second from the beginning of the metronome (arrow M), the α-waves increased markedly in their amplitude until they were suppressed at about the 19th second.

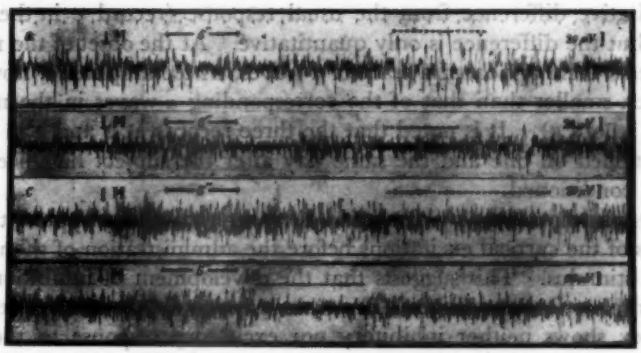

Fig. 3. In all oscillograms the arrow M indicates the start of the metronome beats. Upper three oscillograms are referred to different excitation levels of one and the same subject. The excitation level is highest in a and lowest in c, while the intermediate case is represented by b. In spite of the absence of the unconditioned stimulus, the conditioned excitatory waves are to be seen under the broken line in all three oscillograms. The undermost oscillogram show an example of disinhibition. Upon the electric bell (arrow B), there appeared a long lasting train of β-waves as indicated by the broken line.

The suppression of the so augumented α-waves can be regarded as a conditioned excitatory response of the cortex. Thus, the cortical excitation is delayed by the cortical inhibition induced by the conditioned stimulus. However, such a type of the potential pattern was obtained only when the subject was in a normal psychical state. If the subject was so much excited for some reasons, known or unknown, that his electroencephalogram showed the indications of cerebral excitation such as "excitation potentials" or β-waves, the effect of the conditioned stimulus appeared in a different form. Examples are shown in a and b of Fig. 3. In a, before the administration of the metronome marked "excitation potentials" are seen, which are reduced at the onset of the metronome and appear again as indicated by a broken line in the record. In this case the reduction of the "excitation pot." at the onset of the metronome must be an expression of "inhibition of delay." Judging from the time of the second appearance of the "excitation potentials," it must be a conditioned excitatory response. As stated above, the conditioned excitatory response is usually α-reduction and β-augumentation, and the "inhibition of delay" is expressed in α-augumentation. But in a greatly excited state of the brain we see now that the conditioned excitatory response consists of the "excitation potentials," and that the reduction of the "excitation potentials" is caused by the inhibitory process. Fig. 3 b represents an example in which the β-waves are prevailing in the electroencephalogram. In such a case there is

no qualitative difference from the usual response (record c in the same figure), but the difference is only quantitative. At the onset of the metronome beats there appeared a train of α-waves, whose amplitude, however, was much smaller than that of the corresponding α-waves in the normal control (record c). It is noted that the three records a, b and c in Fig. 3 were obtained on one and the same day after the subject had been sufficiently conditioned.

The patterns (a), (b), (c) and (d) show that there appear the indications of the cortical excitations before the administration of the unconditioned stimulus. This suggests that the development of inhibition is as yet incomplete in these stages. From the pattern (g), it will be seen that the cortex shows neither inhibitory nor excitatory response to the conditioned stimulus. Such a circumstance as this was already noticed by Pavlov. He reported that, especially in the case of the delayed conditioned reflex, the once established conditioned reflex is gradually weakened, though the conditioning of the brain is always strengthened by the procedure of reinforcement.

II. Disinhibition.

Excitation suppressed by inhibition must be freed from the control of the latter when an extra-stimulus disturbs the effect of the prevailing inhibition. Such a phenomenon is known as disinhibition. In the present experiment, the ringing of an electric bell was used as an extra-stimulus. The result is shown in Fig. 3 d. The metronome was started at the arrow M and the bell was rung for half a second at the arrow B. Soon after the beginning of the metronome, the α-waves became more regular and increased in amplitude in a slight degree, and there appeared a fairly long lasting train of β-waves upon the ringing of the bell. The train of β-waves of such a long duration should be interpreted as due to disinhibition caused by the extra-stimulus. However, one may argue against the assumption of disinhibition, because an acoustic stimulus like a bell can suppress the α-waves and gives rise to the β-waves under normal conditions of the subject. Therefore, an experiment was made to answer the question whether the effect of the extra-stimulus mentioned above might be understood as an usual depressing effect of an acoustic stimulus or as disinhibition. The result is illustrated graphically in Fig. 4, in which the average amplitude of α-waves (average of successive twenty waves) is plotted against time. The uppermost curve shows the effect of the bell in a delayed conditioned reflex (original record: d in Fig. 4) and the bottom curve represents effect of the bell alone in a non-conditioned state of the subject. The middle curve refers to the effect of the light stimulus accompanied with the metronome. It will be seen from these curves that the effect of the bell during

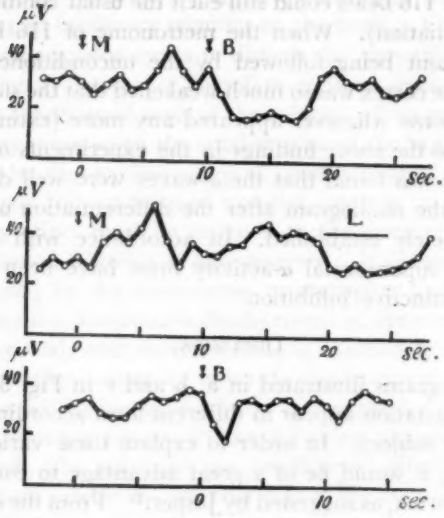

Fig. 4. Changes of average amplitude of successive twenty α-waves. Uppermost curve; Experiment of disinhibition (original record: Fig. 3 d). At the end of ten seconds after the onset of the metronome (arrow M), an electric bell was rang for half a second (arrow B). Middle curve; The electric light (horizontal line L) was administered for five seconds at about 19th second from the beginning of the metronome beats (arrow M). Bottom curve; Control experiment. Under a non-conditioned state of the subject, an electric bell was rang for half a second at the arrow B. The diminution of the average amplitude is only temporary in this case.

the conditioned reflex lasts much longer than that in the non-conditioned state of the subject. Such a long lasting effect of an acoustic stimulus was never seen under normal conditions of the subject. Therefore the effect should be explained by disinhibition, but not by the usual depressing effect of an acoustic stimulus.

III. Generalisation, Differentiation and Experimental Extinction.

At a later stage of conditioning in which the potential pattern (g) was not yet observed, some experiments were made on generalisation, differentiation and experimental extinction. When the frequency of the metronome beats was slightly changed, for instance from 116 to 120 or to 110 per minute, the cortical response was found to be the same as shown in the pattern (f) (generalisation). But when such stimuli were applied successively several times, the cortex ceased to respond to the stimuli, while

the metronome of 116 beats could still elicit the usual conditioned potential changes (differentiation). When the metronome of 116 beats was given many times without being followed by the unconditioned stimulus, the conditioning of the cortex was so much weakened that the stage was reached in which no response whatever appeared any more (extinction).

In addition to the above findings in the experiments of differentiation and extinction, it was found that the α-waves were well developed in the whole extent of the oscillogram after the differentiation or the extinction had been completely established. In accordance with Motokawa's interpretation this supernormal α-activity must have been caused by the differential or extinctive inhibition.

Discussion.

Three oscillograms illustrated in a, b and c in Fig. 3 show that both inhibition and excitation appear in different form according to the mental conditions of the subject. In order to explain these varieties in terms of brain physiology, it would be of a great advantage to consider the excitation level of the cortex, as suggested by Jasper.[4] From the electrophysiological point of view, the excitation level may be characterized by the following potential patterns: 1) "excitation potentials," 2) β-waves, 3) a mixture of α and β-waves, 4) α-waves of increased amplitude. These four patterns may be considered as arranged in order of the strength of excitation. As Motokawa emphasized, the ordinary psychic passive state of the subject is regarded as corresponding to the third level of excitation and does not represent the lowest level of excitation. Now let us consider the normal response represented by Fig. 3 c. In this case it is obvious that before the application of the conditioned stimulus the brain was at the third level of excitation. Upon the conditioned stimulus the state of the brain was shifted from the third to the fourth level. Thus, the inhibitory process induced by the metronome caused the downwards displacement of the excitation level. The conditioned excitatory response, which appeared 20 seconds after the beginning of the metronome, is a shift of the reversed direction. When the cortical excitation of the first level prevailed before the experiment (Fig. 3 a), it was shifted to the second level by the action of the inhibition. In this case the cortical excitation was raised again to the first level in the conditioned excitatory response. As to the case represented by b in Fig. 3, the potential patterns may be explained similarly. In this manner the above mentioned three cases can be understood from one and the same standpoint, though the type of response is aparently different from one another. Generally it can be said that the excitation level is displaced downwards by the inhibitory process and upwards by the excitatory process.

As the inhibitory process is caused by the conditioned stimulus, the latter must act as an inhibitory stimulus on the brain. On the other hand, the unconditioned stimulus serves to disturb the prevailing inhibition and causes the excitatory process. Therefore it is an excitatory stimulus at least in the well conditioned state of the subject. This principle, however, does not hold any more when the conditioning of the brain is not yet complete. At least in the earliest stage of conditioning, "excitation potentials" appear upon the conditioned stimulus (pattern (a) or (b) in Fig. 1). So an excitatory effect must be assigned to the metronome. The effect of the unconditioned stimulus is also reversed; it causes the "excitation potentials" elicited by the metronome to disappear and gives rise to β-waves. This means a downwards displacement of the excitation level from the first to the second, and makes a striking contrast to the fact that the unconditioned stimulus causes an upwards displacement of the excitation level when the conditioning of the brain is complete. We can now formulate the conditioning process in the following manner: As the conditioning proceeds, the effect of the conditioned stimulus becomes less excitatory and more inhibitory, whereas that of the unconditioned stimulus becomes less inhibitory and more excitatory.

Summary.

A delayed conditioned reflex was established in man with a metronome as a conditioned stimulus and with an electric light as an unconditioned one. The interval between the beginning of the metronome and the administration of the electric light was twenty seconds. Changes in electroencephalograms were studied in the course of the establishment of the conditioned reflex.

1. As the conditioning proceeded, cortical responses to the combination of the conditioned and unconditioned stimulus changed in a characteristic manner.

2. When the delayed conditioned reflex was completely established, the amplitude of α-waves increased at the onset of the metronome. In spite of the absence of the unconditioned stimulus the so augumented α-waves were suppressed and β-waves appeared at about the 20th second from the start of the metronome beats. The augumentation of the α-waves is an expression of the internal inhibition developed during the delayed conditioned reflex, and the suppression of the α-waves and the appearance of the β-waves represent the conditioned cortical excitation.

3. An extra-stimulus inhibited the effect of the inhibition induced by the conditioned stimulus and elicited a long lasting train of β-waves (disinhibition).

4. Experiments on generalisation, differentiation and experimental

extinction were carried out when the conditioning was complete. After the differentiation or extinction had been established, it was found that α-waves were well developed in the whole extent of the oscillogram. This phenomenon must be attributed to the differential or extinctive inhibition.

5. The nervous mechanism underlying excitation and inhibition was discussed on the basis of experimental evidence.

Finally I express here my hearty thanks to Prof. Motokawa, who furnished me with guidance and helpful criticism in regard to this work.

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The Influence of Oxygen Lack on Brain Waves in Man.

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Introduction.

If the activity of the human cortex is impaired by anoxia, there must be some changes in the electroencephalograms (eeg), for the electrical potential of the cortex can be regarded as a direct index of the cortical activity. A number of investigators[1-5] reported the changes of eeg caused by oxygen lack, but they referred only to such marked changes as would be found by mere inspection of records. However, I have found that much slighter changes of eeg show themselves in such quantitative measures as mean amplitude and mean period of the recorded brain waves. Therefore, in the present paper, I studied much slighter changes of eeg than those dealt with by the above authors, by describing them quantitatively with mean amplitude and mean period.

Experimental.

Method.

Subjects of experiments were four healthy students. They breathed gas mixtures, containing from six to eleven volume per cent oxygen. The content of oxygen in the gas mixtures was accurately determined by means of Haldane's gas analyzer. The recording system of eeg consisted of an usual R-C-type amplifier and Yokogawa's electromagnetic oscillograph. The lead was monopolar, the different lead being attached to the vertex, the indifferent to the ear lobe.

Experimental procedure was as follows. After eeg had been recorded three or four times with an interval of one minute, I made the subject breath the gas mixture for five to seven minutes, and during this time the recording went on as before. After cessation of the gas breathing, the course of recovery was pursued for several minutes.

In my experiment the mean period means the time occupied by one hundred successive waves, divided by 100. The mean amplitude, i.e. the

arithmetic mean of one hundred waves, was measured by Motokawa's method,[6] which yielded accurate results and saved much time.

Results.

In the first place, I will deal with the results obtained under the lowest oxygen tension applied. An example is shown in Fig. 1. The record

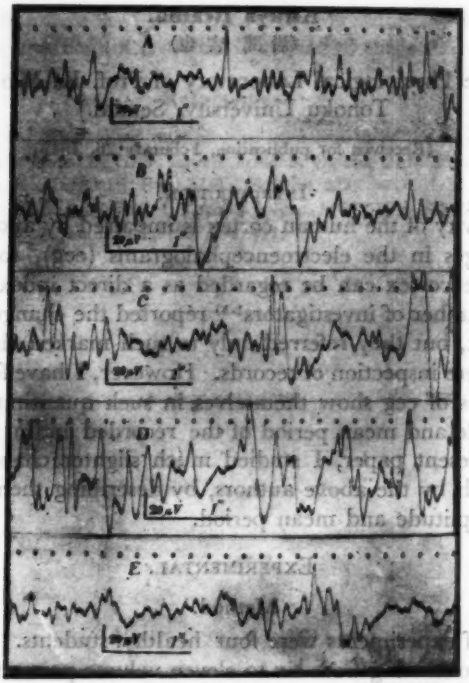

Fig. 1. Eeg of a subject who breathed a gas mixture containing 6.1 per cent oxygen for six minutes. (A) Before the gas breathing; (B) one minute, (C) three minutes, (D) six minutes after the beginning of the breathing (E) four minutes after the end of the breathing.

was taken from a subject who breathed a gas mixture, containing 6.1 per cent oxygen, for six minutes. This oxygen tension corresponds to high altitude of 9,100 m (30,000 feet). In this figure, A indicates eeg in the normal state, and B, C, and D show those during the gas breathing. Within a minute after the beginning of the gas breathing, irregular deflection of the base line and rapid waves of small amplitude appeared (Fig. 1 B). As the gas breathing was continued, the small rapid waves disappeared and groups of large slow waves (∂-wave) were frequently observed (Fig. 1 C,

recorded three minutes after the beginning of the gas breathing). The groups of slow waves appeared more and more frequently, and at the final stage of the gas breathing, the record consisted of almost only δ-waves (Fig. 1 D). Soon after the gas breathing, the δ-waves were replaced by irregular rapid β-waves, and rhythmical α-waves were scarcely seen at this stage of recovery (Fig. 1 E, recorded four minutes after the end of the gas breathing). The α-waves appeared more regularly as the recovery proceeded, and it took over ten minutes to establish the normal pattern of eeg.

The changes of the electrical pattern in the above example can be expressed quantitatively with the mean amplitude and mean period (Fig. 2).

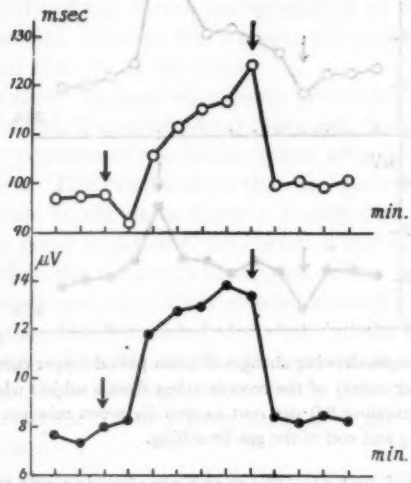

Fig. 2. Graphs showing changes of mean period (upper curve) and mean amplitude (lower curve) of the records shown in Fig. 1. Arrows indicate beginning and end of the gas breathing.

It will be seen that the increase in the mean amplitude ran parallel with that of the mean period. At the end of the gas breathing, the mean period amounted to about 130 msec., and by this time the mean amplitude increased by about eighty per cent of the normal value. It is to be noted that one minute after the beginning of the gas breathing there took place a temporary decrease in the mean period. This decrease in the mean period corresponded to the appearance of the rapid waves shown in Fig. 1 B.

The changes of eeg obtained under a slighter degree of oxygen lack than in the above experiment, were not so greatly different from those of

the above example, except more gradual development of changes. In the following experiment, a subject breathed a gas mixture of 9.0 per cent oxygen for seven minutes. This oxygen tension corresponds to high altitude of 6,500 m (21,500 feet). In this case, the measurement of the mean amplitude and mean period revealed such important features, as will be seen in Fig. 3. One minute after the beginning of the gas breathing not

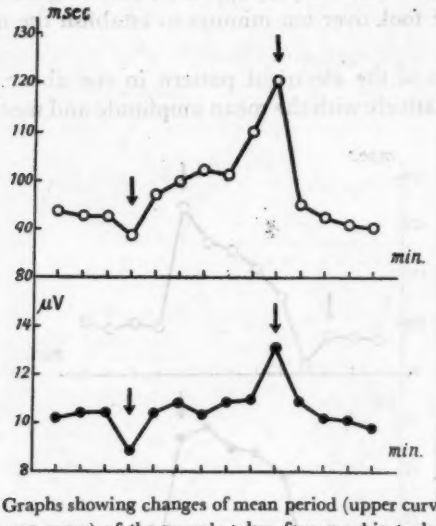

Fig. 3. Graphs showing changes of mean period (upper curve) and mean amplitude (lower curve) of the records taken from a subject who breathed a gas mixture containing 9.0 per cent oxygen for seven minutes. Arrows indicate beginning and end of the gas breathing.

only the mean period but also the mean amplitude were markedly reduced. Then the mean period increased gradually to reach 120 msec. at the final stage of the gas breathing. The mean amplitude, after the temporary depression, rose to the same level as before the gas breathing within one minute, and thereafter remained almost constant for a few minutes till it rose again toward the end of the gas breathing. The increase of the mean amplitude, however, was less marked and only thirty per cent of the normal value. The curves in this figure show that the increase of the mean period took place a little earlier than that of the mean amplitude.

Discussion.

Similar changes as in my experiment, i.e. the decrease in the amplitude of α-waves and the appearance of small fast waves at an early stage of oxygen lack were also noted by Leyman et al,[4] who expressed these

changes by a term "flattning." The authors tried to attribute the "flattning" to the drowsiness of the subjects. But it is scarcely plausible, because the changes occurred within very few minutes after the beginning of oxygen lack and were sometimes observed in the subjects who did not feel drowsy.

In animal experiments it is well known that the activity of the central nervous system is enhanced by a slight degree of oxygen lack (Winterstein[7]). If this is the case also with human being, we may suppose that the "flattning" is an indication of a temporary enhancement of the cortical activity at least at the beginning of oxygen lack.

As shown above, the rapid waves prevailing in earlier stages of oxygen lack were soon replaced by the slow large δ-waves. This means that the supernormal activity of the cortex corresponding to the "flattning" becomes now subnormal, because the δ-waves are generally thought to indicate a decreased activity of the brain.

Sugar & Gerard[8] showed that brain potentials of a cat could be abolished by occulsion of arterial blood flow, and that potentials of phylogenetically newer portions of the brain system were abolished earlier than those of older ones. This results show that the more highly differentiated a region of the brain is, the more easily it is subjected to oxygen lack. If this relation holds good in general, the cortex of the human brain must be impaired earlier than the subcortex in oxygen lack. In all cases I investigated, the respiratory and circulatory systems showed an enhanced activity with accelerated pulse and breathing, while the cortical activity was lowered as measured by eeg. Since the centres for respiration and circulation lie in the subcortex, the above findings are evidence that the human subcortex remains supernormal for a fairly long time after the onset of decreasing activity of the cortex.

On the other hand it is reported that cats or rabbits, subjected to oxygen lack, died within a few minutes after the appearance of any marked changes in eeg, perhaps owing to the paralysis of subcortical centres (Bremer,[2] Huzimori[5]). My results are, however, quite different from those of the animal experiments; in lower animals the impairment of the cortex is followed by the paralysis of the subcortex within a shorter interval than in human being. In other words there is a greater difference of resistance to oxygen lack between both regions of the human brain.

The above results show that eeg is one of the most sensitive indicators of the oxygen lack of the cortex, especially in man, and that the mean period is a more sensitive measure than the mean amplitude, because the former increases earlier than the latter, especially when oxygen lack is of a slight degree.

With respect to the irregularities of amplitudes, periods and wave

form, eeg taken during the recovery stage resemble those observed by Motokawa et al[9] in the state of mental fatigue. This fact suggests that there exists a close relationship between mental fatigue and oxygen lack of the human cortex.

SUMMARY.

Electroencephalograms (eeg) were recorded in normal subjects, who breathed gas mixtures containing from six to eleven volume per cent oxygen for five to seven minutes. Changes of eeg were expressed with mean amplitude and mean period obtained from the recorded brain waves.

1. Under a high degree of oxygen lack, following changes of eeg were observed. Within a minute after the beginning of the gas breathing, small rapid waves appeared, and corresponding to this change, the mean period was temporarily diminished. Two or three minutes later slow large δ-waves appeared in groups, and the mean amplitude and mean period increased rapidly hand in hand. In the final stage of the gas breathing eeg consisted of almost only δ-waves.

2. In oxygen lack of a slighter degree potentials were markedly suppressed at an early stage of the gas breathing, and thereafter the mean period increased prior to the mean amplitude.

3. Soon after the cessation of the gas breathing, δ-waves disappeared, but the normal pattern of eeg was not recovered for a long time. Eeg at the recovery stage were found similar to those obtained in mental fatigue.

Prof. MOTOKAWA furnished guidance and helpful criticism, for which I express here hearty thanks.

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Notes on Uncus gyri hippocampi in the Gibbons.

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Introduction and Materials.

In spite of intensive researches into the detailed anatomy of the hippocampal formation in recent decades, there is still much to be learned regarding the morphology, structure and function of the uncus. Although the literature dealing with the human uncus may be said to be voluminous, there has been, with a few exceptions, no intensive study of this region in the monkey brain. The comparatively detailed studies of the uncus in monkey brain are those of Retzius[1] (Cynopithecus), Levi[2] (Orang Utan), Rose[3] (Cynocephalus hamadryas), Völsch[4] (Lemur mongoz, Macacus rhesus) and Altschul[5] (Macacus rhesus, Troglodytes niger).

It is clear from a review of literature that there remain many gaps in our knowledge as to the morphological and structural changes in the uncus of monkies from the lower to higher classes. It is high time that these gaps were filled by a systematic and detailed study which would aid greatly in the difficult task of interpreting the maze of the hippocampus as it is known today. Fortunately there are a fine series of brain specimens of various monkies in this laboratory, and so we have undertaken to investigate them.

The present paper records only the result of investigations dealing with the uncus in the two species of gibbon: Hylobates lar L. and Hylobates leuciscus E. Geoffr. This material consists of complete serial frontal sections (each 35μ thick), which are stained by the Pal-Weigert method and counterstained with Paracarmine.

Hylobates lar L.

Left: In this ape the uncus is remarkably well developed and its caudal-part of 2.24 mm. is projected backward as a socalled free uncus, separating from the hippocampus proper and the fimbria hippocampi.

The appearances of the hippocampal formation are represented in a

purely schematic manner in the accompanying figure. The most interesting and conspicuous feature of the hippocampal formation is that, as is demonstrated in the figure 1 E, the free uncus shows the three-leaved type, being divided into a dorsomedial, a dorsolateral and an intermedial part. The first two parts are respectively composed of a pair of the stratum granulosum and the layer of pyramidal cells. In these the str. granulosum is represented as a V-shaped band of deeply stained cells, and the layer of pyramidal cells is present as a hook-like curved lamina of closely arranged cells. It will be further noted that the str. granulosum and the pyramidal layer are so arranged that one crooked tip of the latter lies between the divergent limbs of the former. In the intermedial part the granular band is wanting, and the pyramidal cells are seen in this part to be arranged in a radiating manner from the center to the periphery. As it is traced on dorsally these pyramidal cells gradually give place to both the layer of pyramidal cells of the dorsomedial part and those of the dorsolateral part described above.

Laterodorsal to the uncus, but separated from it by a wide fissura fimbriodentata, lies the gyrus dentatus accompanied by the hippocampus proper. The str. granulosum of this gyrus is shaped somewhat like a sac with the hilus directed dorsally and its basal lamina showing a sharply jagged indentations. On the other hand the layer of pyramids of the hippocampus proper, which springs ventrally from the subicular region, is prolonged dorsal-ward through the hilus of the granular sac into a layer of comparatively scattered polymorph cells (Nucleus fasciae dentatae of Smith[6]).

With the view of determining the relationship between the uncus and the hippocampus proper, let us trace the sections forward and backward, beginning from the level of Figure 1 E. As one examines the series of sections backward (E-A in the Fig. 1), one finds the following appearances: I) The two V's of str. granulosum in the dorsolateral and dorsomedial parts of the uncus gradually approach one another until they combine with each other, producing the common W-shaped band of granules (Fig. 1 B). As it is traced farther backward this W is transformed at first into an arch opening dorsally (Fig. 1 A), and then becomes reduced to a sharply defined, elliptical mass of closely packed cells, which falls entirely in front of the caudal pole of the free uncus. 2) Both layers of pyramidal cells of the dorsomedial and the dorsolateral parts approach each other centrally and combine to form X-shaped band (Fig. 1 D). At a short distance from this section it forms two arches of pyramids as a result of disappearance of the united portion described above, the upper arch extending between the two granular V's and the lower arch remaining in the intermedial part (Fig. 1 C). Upon further sectioning posteriorly, the lower arch

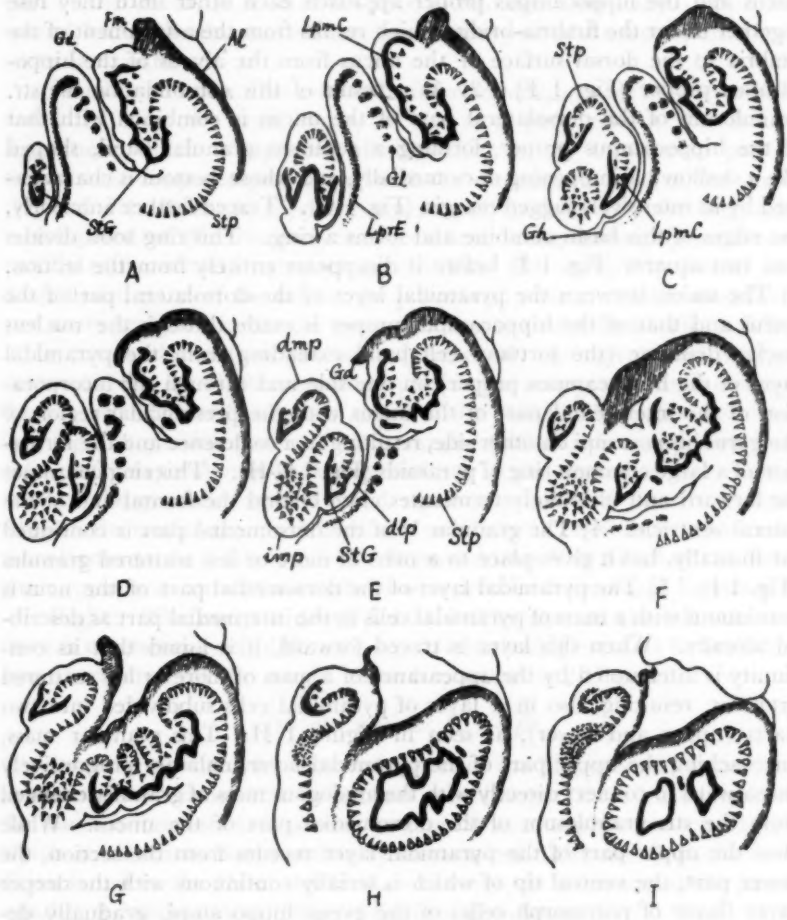

Figure. 1. Hylobates la L.

Al, Alveus; Fm. Fimbria; Gd, Gyrus dentatus; Gh, Gyrus hippocampi; Gl, Glomerulus of granular cells; LpmC, Layer of polymorph cells; LprE, Lamina principalis externa; StG, Stratum granulosum; Stp, Stratum cellul. pyramid.; U, Uncus:-dlp, dorsolateral part; dmp, dorsomedial part; imp, intermidial part.

gradually disappears (Fig. 1 B), while the upper arch remains (Fig. 1 A) though diminished in size.

If the hippocampus is now traced forward in the series of sections (E-I in the Figure 1), it is observed as follows: I) The dorsolateral part of the

uncus and the hippocampus proper approach each other until they fuse together under the fimbria-bridge which results from the attachment of the fimbria to the dorsal surface of the uncus from the alveus of the hippocampus proper (Fig. 1 F). 2) As a result of this consolidation the str. granulosum of the dorsolateral part of the uncus is combined with that of the hippocampus proper, forming a common granular band, shaped like a shallow basin opening dorsomedially, and whose bottom is characterized by its intensively jagged margin (Fig. 1 G). Traced farther anteriorly, the edges of this basin combine and forms a ring. This ring soon divides into two squares (Fig. 1 I) before it disappears entirely from the section. 3) The union between the pyramidal layer of the dorsolateral part of the uncus and that of the hippocampus proper is made through the nucleus fasciae dentatae (the tortuous cell band extending from the pyramidal layer of the hippocampus proper) on one side and through the incorporation of the intermedial part of the uncus with the presubicular region of the gyrus hippocampi on other side, resulting in a coalesence and the formation of a large common ring of pyramids (Fig. 1 G-H). This ring continues far forward and ultimately terminates close behind the frontal end of the lateral ventricle. 4) The granular V of the dorsomedial part is continued far frontally, but it gives place to a mass of more or less scattered granules (Fig. 1 I). 5) The pyramidal layer of the dorsomedial part of the ucus is continuous with a mass of pyramidal cells in the intermedial part as described already. When this layer is traced forward, it is found that its continuity is interrupted by the appearance of a mass of more or less scattered granules, resulting also in a layer of pyramidal cells subdivided into two parts (upper and lower), as seen in Figure 1 H. This granular mass, encroaching the upper part of the pyramidal layer, enlarges progressively dorsalward to connect directly with the analogous mass of granules changed from the str. granulosum of the dorsomedial part of the uncus. While thus the upper part of the pyramidal layer recedes from the section, the lower part, the ventral tip of which is serially continuous with the deeper layer (layer of polymorph cells) of the gyrus hippocampi, gradually decreases its cellular components.

On reviewing the above observation it will be evident that the str. granulosum of the dorsolateral part of the uncus is connected caudally with the str. granulosum of the dorsomedial part of the uncus on one side, and it is anteriorly continuous with the str. granulosum of the hippocampus proper on the other. The same description is applicable also to the layer of pyramidal cells. Accordingly it may be described as follows: The hippocampal formation proceeds (as the hippocampus proper) anteriorly so long as it knocks against the frontal end of the lateral ventricle. After having there recurved medially, it traces backward (as the dorsolateral

part of the uncus) in a short distance to fold again medially. After this second folding it advances (as the dorsomedial part of the uncus) anteriorly on its last course.

In addition to the above-said, the following features are to be especially noted: I) The str. granulosum, for the most part, exhibits the distinct jaggedness, which is especially distinguished at the transitional portion between the hippocampus proper and the dorsolateral part of the uncus (Fig. 1 F-H). 2) In the hippocampus proper, the layer of polymorph cells is composed of the comparatively scattered cells, arranging somewhat parallel to the accompanying str. granulosum. But in the dorsolateral and dorsomedial parts of the uncus, the polymorph cells are rather disorderly and more scattered inside the accompanying granular band (Fig. 1 B-D). 3) In the caudal sections passing approximately through caudal third of the uncus (Fig. 1 A-D), between the hippocampus proper and the free uncus, there is pushed dorsally a mass of cortex of the presubicular region, resulting in a high tongue-like process, in the margin of which there is an area consisting of comparatively packed granules. In the lateral margin of the process, the granular area is found separating into 5–6 discrete groups, while in the medial margin it is present as a layer. The former is probably homologous to the " Glomeruli der Körnerschichte " of Altschul,[7] and the latter apparently corresponds to the lamina principalis externa of Economo.[8]

Right: The uncus is developed as well as that on the left side and there is no essential side-difference worth mentioning not only in form but also in structure of the uncus. The free uncus is 2.17 mm.

Hylobates leuciscus E. Geoffr.

In so far as the twice-recurved course of the hippocampal formation, producing the uncus, and its general morphology (three-leaved type of the uncus in cross-section) as well as general structure are concerned, the Hylobates leuciscus does not display any marked difference from the condition found in the Hylobates lar L.; however, in reference to the particularity, it exhibits some different features as follows:

1) The free uncus is measured 3.29 mm. on the left side and 2.03 mm. on the right side.

2) The str. granulosum not only of the uncus but also of the hippocampus proper is characterized generally by somewhat smooth and slight waves (it does not show such sharp jaggedness as in the Hylobates lar L.).

3) The layer of polymorph cells (nucleus fasciæ dentatæ) of the hippocampus proper is composed of closely packed cells, which are arranged very regularly parallel to the granular band.

4) In this ape the socalled " glomeruli " of granules is entirely absent

in the presubicular region, but the lamina principalis externa is distinctly present in the medial margin of the process, which is projected dorsally between the hippocampus proper and the free uncus in such form as is seen in the Hylobates lar L.

Summary and Discussion.

1) In the Hylobates lar L. and Hylobates leuciscus E. Geoffr the uncus are found generally well developed.

The sagittal diameter of the free uncus will be summarized as follows:

	Left	Right
Hylobates lar L.	2.24 mm.	2.17 mm.
Hylobates leuciscus E. Geoffr.	3.29 mm.	2.03 mm.

2) One of the most striking characters of the uncus in the gibbons is the fact that the free uncus, in its cross-section, is represented as a three-leaved type, divided into a dorsomedial, a dorsolateral and a intermedial part. The intermedial part of the uncus is a backward elongated portion of the presubicular region of the gyrus hippocampi. The other two parts are the recurved continuation of the hippocampus, which repeats twice recurvation in order to perform the uncus. This recurvation of the hippocampus has been briefly described only by Völsch in the investigation dealing with the amygdala of the Lemur mongoz.

3) The hippocampal formation, throughout its whole recurved course, carries with it always the str. granulosum (Fascia dentata) and the layer of pyramidal cells. This fact would appear to indicate that the str. granulosum, through its developmental career, moves always in harmony with the layer of pyramidal cells. But this supposition is not compatible with that of Altschul[9][10] and Levi, who assumed that the hippocampus and the fascia dentata developed severally at different times and took their respective ways in the uncus. But their claim is hardly justified, since we have completely failed to find such a trace in the uncus of the gibbons. At any rate this point remains yet to be proved in future.

4) The shape of the str. granulosum (fascia dentata) seems to vary more or less according to species of animals. In the Hylobates lar L., the granular band is characterized by its intensely jagged type, while in the Hylobates leuciscus E. Geoffr it shows the smooth type. It is to be emphasized that the str. granulosum, in our two gibbons, is demonstrated almost throughout the extent of the uncus and its caudal end almost reaches the caudal pole of the free uncus. This is a very important feature in view of the statement of Altschul that the longer caudal extension of the fascia dentata in the uncus would appear to indicate the higher development of the uncus, and that the development of the uncus, phylogenetically, may be in harmony with that of the brain as a whole

5) In the Hylobates lar L. the granular area in the presubicular region is sharply differentiated, separating into several glomeruli, while in the Hylobates leuciscus E. Geoffr it is wanting. These glomeruli may be identified as the homologue of the pars glomerulosa areae presubicularis glanulosæ of Economo and the "Glomeruli der Körnerschichte" of Altschul.

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A Modification of Cardiotachograph.

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Since I reported of an instrument, called *Cardiotachograph*, for the continuous recording of instantaneous changes in pulse rate[1], I tried to improve this instrument so as to give more clear curves not obscured by numerous vertical lines. An idea for such a modification is described here.

The principle and the circuit diagram (Fig. 1) are similar to those of my original *Cardiotachograph*, except: the final recording is performed, without amplifier, by a galvanometer which responds rather slowly (period of vibration, optimally damped, 5 seconds, sensitivity $1{\cdot}3\times10^{-9}$ amp). In the photographic record are described, synchronous with cardiac beats, small spikes moving up or down according to the instantaneous changes in heart rate (Fig. 2). Theoretically speaking, the curve may have some time-lags behind the quick changes in cardiac rhythm; such lags, how-

ever, are insignificant for practical purposes. Even the respiratory arrhythmias are recorded quite distinctly.

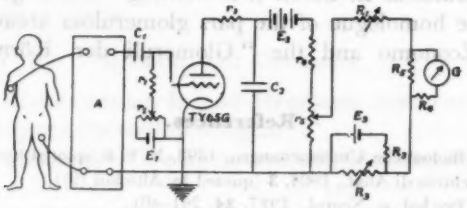

Fig. 1. Circuit diagram of new type of cardiotachograph.

A :	amplifier of heart action potential.		
G :	galvanometer for recording cardiotachogram.		
C_1:	0.1 μF, C_2: 4μF.	E_1:	22.5 V, E_2: 100 V, E_3: 6 V.
r_1 :	2MΩ.	R_1:	500 KΩ.
r_2 :	3KΩ	R_2:	1000Ω.
r_3 :	200Ω.	R_3:	1000Ω.
r_4 :	250KΩ.	R_4 and R_5:	galvanometer shunt.
r_5 :	200KΩ.		

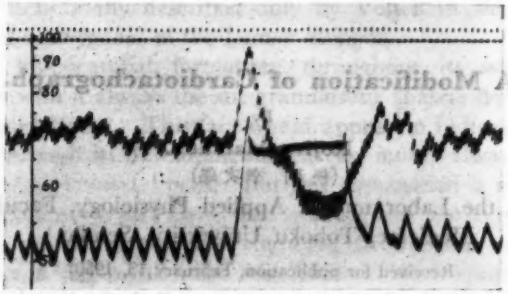

Fig. 2. Record by the new type of cardiotachograph. Continuous record of changes in cardiac rhythm of man on holding the breath after deep inspiration. Upper: time marking in two-second interval. Middle: cardiotachogram, pulse rate per minute can be read in the scale on left. Lower: respiration, inspiration upward.

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Treatment of Pulmonary Tuberculosis by Monaldi Transthoracic Cavity Suction Drainage with Thoracoplasty.

By

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It is widely known, that the Monaldi procedure is one of the surgical treatments for pulmonary tuberculosis. The chief characteristics of this procedure are: (1) the operation needs only a simple technique; (2) The post-operative reaction is quite light; (3) Some cavities contract clearly within from 2 to several months after treatment; (4) The inner wall of the cavity is purified and the caseous layer is shed and discharged; the tuberculous granulation tissue is mostly replaced with non-tuberculous granulation tissues; the tubercle bacilli are much reduced or disappear, etc.

If, however, this treatment is stopped and the suction-catheter is taken off, the residual cavity which disappeared from radiogram begins to re-open with the elapse of time, and simultaneously the clinical symptoms often get worse. This is quite natural when it is considered from the principle of the transthoracic cavity suction treatment. The mechanism of the cavity contraction in this treatment is: As the result of continual decline of the intracavital pressure, there occurs a emphysematic inflation of parenchym around the cavity wall, and the chest-wall and mediastinum are retracted towards the cavity; therefore, when the treatment is stopped, those tissues and organs which were under the outer control begins to return to their former places, and consequently the cavity begins to re-open itself. And, one of the reasons why the residual cavity re-opens after the treatment, is because the lesions cannot get rest owing to the natural breathing of the thorax and this hinders the anatomical healing.

We made investigations on 16 cases in which only the Monaldi procedure was adopted with the following results, 9 cases of cavity re-opening which returned to their former size could be recognized, 6 cases were not tracted and there was only one case with no cavity re-opening. In the

majority of the cases re-opening was recognized several months after the procedure was stopped. Therefore, it is readily noticed that this procedure alone is not effectual, but that it will be more effectual when adopted with such other surgical treatment as can prevent the cavity re-opening.

As means for preventing the cavity re-opening after the adoption of this procedure there are:

(1) To let the bronchi leading to the cavity desolate by medical action or electric coagulation and to cause an obstraction on the scar-tissue (Coryllos a. Ornstein[1]).

(2) To fill the opened cavity with the pedicled muscle flap (Coryllos a Ornstein, Aoyagi[2]).

(3) To adopt thoracoplasty along with the Monaldi procedure.

Burnahd,[3] Franken, Kremer,[4] Kupka[5] had already proposed that the co-adoption of the thoracoplasty should be either as a means for preventing the cavity re-opening after the adoption of the Monaldi procedure, or when the cavity was not closed completely by the adoption of the Monaldi procedure. Monaldi[6] himself suggested the co-adoption of his anterolateral thoracoplasty and the transthoracic cavity suction drainage. Ebina[7] of our Institute in his special report on the Monaldi procedure at the Meeting of the Japanese Association for Tuberculosis held in Tokyo in 1942 stated that treatment by the Monaldi procedure alone is not satisfactorily effectual, but that co-adoption with some surgical treatment is quite effectual. Since then, we have been experimenting on the adoption of the transthoracic cavity suction drainage along with the thoracoplasty and phrenic paralysis. Thus, especially, for such a giant cavity which, from our experience, is considered difficult for us to expect complete closing simply by adopting the thoracoplasty, however complete a removal of the upper ribs, including a portion of the corresponding transverse process with extensive extrafascial apicolysis may be done, we have been adopting a positive treatment by the Monaldi procedure with thoracoplasty. We have 24 cases of this kind of co-adoption. The following is our results.

The process of the cavity contraction in the Monaldi procedure was investigated by the serial radiograms experimentation, in which it was found that the contraction differs according to the size and shape of the cavity, to the disposition of the cavity wall, to the existence of the elastic pulmonary parenchyma around the cavity, or to the condition of the leading of the drainage bronchi. For instance, the cavity which is within 5 cm in diameter, almost round in shape, thin-walled and elastic, and which shows, moreover, an elastic pulmonary parenchyma around the cavity, was found to contract wonderfully well in a short course of time when the Monaldi procedure was adopted and it thus disappears from the radiogram. However, in a cavity which is over 5 cm in diameter, deformed, thick-walled

and hard, or to which thick drainage bronchi lead, contracted only in some degree but did not close completely even when the Monaldi procedure was adopted. Generally speaking, the cavity was found to contract quite remarkably within from 2 or 3 months to several months after the adoption of the Monaldi procedure, but the contraction hereafter was slower and slighter.

And, in most cases, it was realized that by the continual adoption of the Monaldi procedure during several months the inner wall of the cavity was purified more or less highly, and the fluid sucked by the suction catheter became serous, slightly pussy, or sero-haemorrhagic, and became only 1–5.6 ml in quantity, and in the meantime the tubercle bacilli within it also became negative in the microscopic examination. An autopsy was undertaken on a woman who died of other disease about 10 months after the adoption of the Monaldi procedure in our Institute, and a pathological examination was done anatomically on the cavity wall, and it revealed almost no caseous necrotic substance on the inner wall of the cavity, which, however, was covered with non-specific granulation tissue with thick collagenous fibre under it, and was purified in general with merely a few cell infiltrations on the whole layer with no traits of tubercle nodosity (Fig. 1).

Thus, at the time when, by adopting the Monaldi procedure, the cavity was contracted as much as possible and the inner wall of the cavity was purified, we adopted the thoracoplasty side by side.

Fig. 1. N. K., aged 25, f. Cavity wall after 10 months from Monaldi procedure.

Fig. 2. (Case 5) Y.Y. aged 26, m. Giant cavity (11.5×6 cm) in left upper lobe. (10.4 1944) Sputum Gaff. VIII.

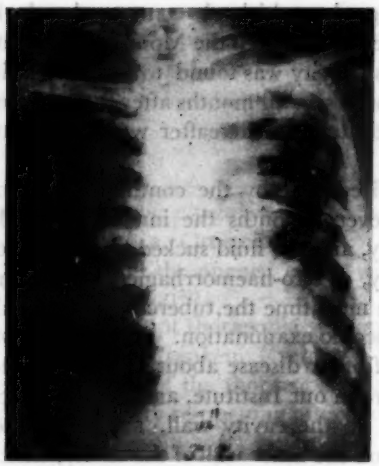

Fig. 3. (8.3 1945) 5 months after the Monaldi procedure in the left cavity.

Fig. 4. (8.3. 1945) Lipiodol injection in the residual cavity through the suction catheter. Notice lipiodol in the residual cavity and in the draining bronchi.

Fig. 5. (4.20 1946) 5 months after the co-adoption of the upper thoracoplasty (7-ribresection) Sputum negative.

Fig. 6. The condition of the residual cavity after the co-adoption of the upper thoracoplasty. No recognition of the residual cavity.

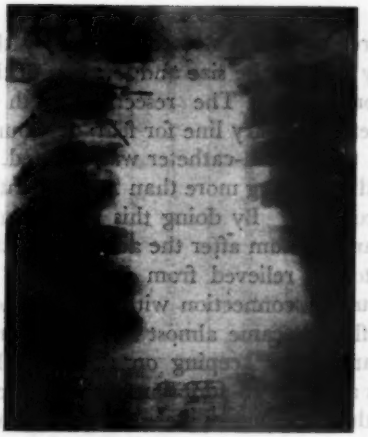

Fig. 7. (Case 16) K.T. aged 35. m. Giant cavities in both upper lobes, (left 6.5×5cm, right 7×5.5cm). Sputum Gaff. VIII.

Fig. 8. Tomogram (7 cm from behind).

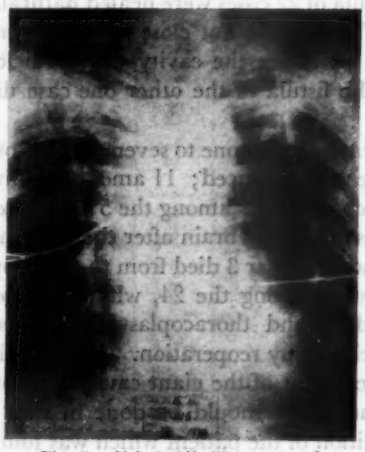

Fig. 9. (4.1. 1944). 20 months after Monaldi procedure, with no recognition of the residual cavities.

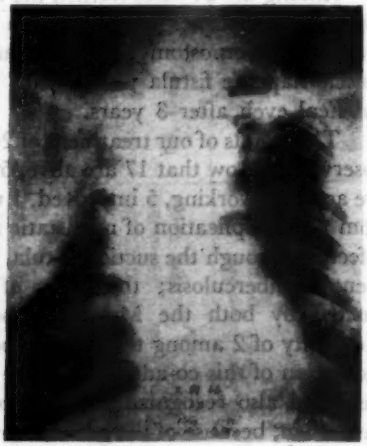

Fig. 10. (11.3. 1946). Two years after the co-adoption of thoracoplasties on both sides. Sputum negative, actually working.

In all the cases as the suction-catheter had been in erted a little more mesially than the mammary line between the first and the second intercostal space of the upper front breast, we gave the paravertebral upper

thoracoplasty.

The length and number of ribs removed was not considered by the size and location of the residual cavity but by the size and location of the cavity before the adoption of this procedure. The resection-length of upper ribs was limited within the anterior axillary line for fear of wound infection by the fistula through which the suction-catheter was inserted.

We continued the Monaldi procedure during more than 2 or 3 months even after the adoption of the thoracoplasty. By doing this the patients were found not to suffer from cough and sputum after the adoption of the thoracoplasty and also were found to be relieved from the danger of bronchogenic spread which often occurs in connection with the adoption of thoracoplasty. When the suction-fluid became almost serous or sero-haemorrhagic and very little in quantity by keeping on the Monaldi procedure during more than 2 months after the co-adoption of the thoracoplasty, and then, by investigating whether the sputum increases or not, and whether the secretion stagnates in the cavity or not, even after the suction was suspended during 2 or 3 days, it was realized that such phenomena were not recognized, the suction-catheter should be taken off gradually during 1–2 weeks.

Among the 24 cases studied the fistula of 22 cases were healed naturally with the scar tissue. As the fistula of 1 case did not close even after one year, the cavernostomy was done and by filling the cavity with pedicled muscle flap the fistula was closed. The fistula of the other one case did not heal even after 3 years.

The results of our treatment of 24 cases during one to seven years of our observation show that 17 are alive, 5 dead, 2 untraced; 11 among 17 alive are actually working, 5 improved, 1 unchanged; 2 among the 5 dead, died from the complication of metastatic abscess of the brain after the pyogenic infection through the suction-fistula, and the other 3 died from the development of tuberculosis; the cavity of the 6 among the 24, who were even treated by both the Monaldi procedure and thoracoplasty, reopened; the cavity of 2 among them could be closed by reoperation. We find the indication of this co-adoption in the treatment of the giant cavity first, but we could also recognize that this co-adoption should be done in such a case when, because of the general condition of the patient which was found to be not suitable for an instant thoracoplasty treatment, we, after trying, as a pre-treatment, to make the condition better and making the cavity contract by the adoption or the Monaldi procedure, co-adopt the thoracoplasty of a comparatively smaller scale. Sometimes to the residual cavity after the adoption of the thoracoplasty, the co-adoption of the Monaldi procedure is also done.

TABLE I.

Case Number	Name, Age, Sex	X-ray Sputum findings	Date of the adoption of the Monaldi procedure	Condition of the cavity contraction	Date of the co-adoption of the thoraco-plasty	Outcome
1	S.F. 23 m	6×3 cm thin-walled cavity, on the right upper lobe, Sputum G. VI	8.8, 1941	1.6×1.2 cm, after 3 months	7.18, 1944	Sputum negative, actually working.
2	N.C. 23 f	6×3.5 cm thin-walled cavity on the left upper lobe, G. IX	6.2, 1941	1×1 cm, after 1 year	10.13, 1946	Sputum negative, improved
3	T.N. 23 f	5.5×5 cm thin-walled cavity on the left upper lobe, G. V	4.14, 1946	3×3 cm after 1 year	11.5, 1946	Sputum negative actually working at home.
4	T.S. 25 m	5.5×4 cm thick-walled cavity on the right upper lobe, G. V	3.25, 1946	untraced	3.13, 1947	Died of the complication of metastic abscess of the brain one month after the adoption of the thoraco-plasty.
5	Y.Y. 26 m	11.5×6 cm thick-walled cavity on the left upper lobe, G. VIII	3.5, 1945	6×3 cm after 1 year	12.4, 1945	Sputum negative, actually working.
6	M.Y. 27 m	3.7×2.1 cm thin-walled cavity on the right upper lobe, G. IV	6.4, 1941	1×1 cm after 3 months	12.6, 1944	Sputum negative, actually working.
7	S.K. 27 m	8×5 cm thin-walled cavity on the left upper lobe, G. VIII	11.9, 1942	3×2 cm after 6 months	9.9, 1944	G. IV, unchanged, (cavity re-opened)
8	K.S. 28 m	7×6.5 cm thin-walled cavity on the left upper lobe, G. IX	9.14, 1944	3×3 cm after 3 months	4.12, 1945	Actually working (cavity reopened, but closed after the reoperation)

9	S.K. 28 m	5×3.5 cm thick-walled cavity on the left upper lobe, G. IX	8.10, 1940	1.7×2.5 cm	8.12, 1942	(Cavity-opened), untraced.
10	Y.S. 29 m	7×5.5 cm thick-walled cavity on the right upper lobe, G. VI	10.10, 1942	1.6×1.3 cm after 3 months	7.28, 1944	Sputum negative, actually working.
11	S.M. 32 m	6×5.5 cm thick-walled cavity on the right upper lobe, G. VII	6.28, 1941	0.6×0.6 cm after 1 year	7.28, 1944	Sputum negative, improved.
12	K.M. 33 m	7×3.5 cm thin-walled cavity on the left upper lobe, 8×4.5 cm thin-walled cavity on the right upper lobe, G. VII	(left) 1.16, 1946 (right) 2.20, 1947	untraced	(left) 6.17, 1947 (right) 10, 1947	Sputum negative, improved.
13	K.T. 33 m	7.5×3.5 cm thick-walled cavity on the left upper lobe, G. IX	1.20, 1945	6.5×1.5 cm after 1 year	12.17, 1947	G. I, improved.
14	O.J. 33 m	9×3.5 cm thick-walled cavity on the left upper lobe, G. X	8.3, 1940	5×1.5 cm after 6 months	2.9, 1942	Died.
15	S.T. 34 m	3.5×2.8 cm thick-walled cavity on the right upper lobe, G. VIII	7.7, 1941	1×1.5 cm after 1 year	6.20, 1942	Cavity re-opened, died.
16	K.T. 35 m	6.5×5 cm thin-walled cavity on the left upper lobe, 7×5.5 cm thin-walled cavity on the right upper lobe, G. VIII	(left) 1.26, 1942 (right) 3.13,	0.8×0.8 (in after 6 months 0.9×0.8 cm after 6 months)	(left) 5.18, 1944 (right) 10.25, 1944	Sputum negative, actually working (right cavity re-opened, but closed by re-operation).
17	M.T. 35 m	7.6×4.5 cm thick-walled cavity on the left upper lobe, G. VI	8.28, 1940	4.3×2 cm after 6 months	3.10, 1941	(Cavity re-opened), untraced.

18	M.M. 39 m	7.6×4.5 cm thin-walled cavity on the left upper lobe, G. VIII	12.5, 1940	4.5×1.5 cm after 1 year	9.3, 1942	Sputum negative, actually working at home.
19	H.Y. 39 m	7×4.5 cm thick-walled cavity on the left upper lobe, G. III	9.15, 1945	5.5×2 cm after 6 months	2.22, 1946	Sputum negative, actually working at home.
20	S.B. 39 m	8×5.5 cm thick-walled cavity on the left upper lobe, G. VIII	11.1, 1944	5×1.5 cm after 8 months	7.7, 1945	Sputum negative, actually working.
21	K.S. 41 m	8×4.5 cm thick-walled cavity on the right upper lobe, G. VIII	1.30, 1941		4.22, 1947	Sputum negative, improved.
22	S.T. 44 m	3.5×3 cm thick-walled cavity on the right upper lobe, G. VIII	7.17, 1941	4.5×1 cm after 1 year	6.20, 1942	Died.
23	T.T. 44 m	8×4.5 cm thick-walled cavity on the right upper lobe, G. III	4.25, 1945	4.5×1 cm after 6 months	3.7, 1946	Sputum positive, improved.
24	I.M. 49 m	8×5.5 cm thick-walled cavity on the right upper lobe, G. I	12.5, 1944	4.5×1.5 cm after 1 year	11.5, 1946	Died of the complication of metastatic abscess of the brain 6 months after thoracoplasty co-adopted.

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On the Isolation of a Strain of Penicillia which Possesses a Bacteriostatic Action against Gram-Positive Bacteria and a Property to Produce Penicillin.

By

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Introduction.

Fleming[1] (1929) noted that a fungus growing on a plate in laboratory brought about an inhibition of the staphylococcus around it and produced a very powerful antibiotic substance. According to his research it was shown that the production of this antibiotic substance was not common to all types of penicillia and he named the substance contained in broth filtrate as " penicillin," which inhibited the growth of the common pathogenic bacteria, largely the Gram-positive ones, including gonococci and meningococci, but did not act upon the Gram-negative bacteria including the colon-typhoid-dysentery group. So he suggested its use as an inhibition for the isolation of certain types of bacteria, especially H. influenzae, and in the treatment of bacterial infections. First he believed that in its morphology this fungus was a penicillium and in all its characters it most closely resembled *Penicillium rubrum*. But examination of Fleming's organism by Dr. Charles Thom[2] showed that it was not *Penicillium rubrum*, but a strain closely related to " *Penicillium notatum Westling* " in the *Penicillium chrysogenum Thom* series.

Clutterbuck, Lovell and Raistrick (1932)[3] studied the growth of this fungus in a medium containing inorganic salts only and isolated a pigment, " chrysogenin " which had no antibacterial action. Their culture media contained penicillin, but this was not isolated by them.

Raid (1935)[4] reported a work on the inhibitory substance produced by Fleming's fungus. He did not isolate it, but noted some of its properties. After the studies of Florey et al[5] concerning the isolation of penicillin in a powder form and its application upon the chemical treatment many methods have been devised for obtaining a considerable yield

of penicillin, and for rapid assay of its inhibitory power. From the culture medium a brown powder has been obtained which is freely soluble in water, and showed a considerable antibiotic activity, but it was not yet obtained in a crystalline form.

As above mentioned, there were already many studies on Fleming's penicillin in America and Europe, but when I was searching for penicillin strains during war time (1944), the author was first in this country to isolate a strain of penicillia from contaminated blood-agar, which was identified later as *P. notatum*. The strain—most resembled Fleming's penicillium—produced an antibiotic substance against Gram-positive bacteria which was found very similar to penicillin.

Experimental.

Characters of the Fungus.

A modified Czapek-Dox medium with the addition of peptone was poured in 250 ml. amounts into 500 ml flasks and inoculated with spores of about a 7 day's culture of this fungus grown on a modified Czapek-Dox agar. In two or three days a pellicle, as a white fluffy mass covered the surface of the fluid. When it sporulated further, it became gradually dark green, changing in about two weeks to a dark green felted mass. In a few days' incubation of this fungus a bright yellow color was produced which diffused into the medium. The reaction of medium was found to change initially from pH 7.0 to a slight acidity (pH. 6.0), followed later by alkalinity, finally reaching a pH of 7.8 or even 8.0.

Examinations of the morphology of this penicillium, which I isolated, by Dr. T. Jinbo at my request, elucidated that it was a strain closely related to *Penicillium notatum*.

Cultural Conditions and Rate of Development of Inhibitory Substance in Culture Media.

Various culture media as follows were examined upon the formation of this antibiotic substance. The inhibitory power could be accurately titrated by making serial dilutions of this culture filtrate in agar plates, and by implanting all Petri dishes with the same volumes of staphylococcus-suspension. Then the inhibition could readily be seen by noting the growth of staphylococci on agar plates.

A)

Medium 1: broth medium with 1% glucose.
Medium 2: broth medium with 5% glucose.
Medium 3: broth medium with 10% glucose.

Medium 4: broth medium with 15% glucose.
Medium 5: broth medium with 1% saccharose.
Medium 6: broth medium with 5% saccharose.
Medium 7: broth medium with 10% saccharose.
Medium 8: broth medium with 15% saccharose.
Medium 9: Czapek-Dox medium.
Medium 10: Czapek-Dox broth medium.
Medium 11: Czapek-Dox medium with 0.4% sodium glutamate.
Medium 12: broth medium with 3% glucose.
Medium 13: broth medium with 3% glucose and 0.1% K_2HPO_4.
Medium 14: Czapek-Dox medium with 1% Katayama's peptone.

The reactions of these media were initially slightly acid (pH 5.0). After the inoculation the pH varied from 5.0 to about 4.0 and later gradually became alkaline.

Complete inhibitory powers of this culture filtrate against staphylococci (D-69 strain in our laboratory) were titrated as a 1:50–400 dilution. Further incubation resulted at first in a slight loss and later in a rapid loss of antibiotic activity.

B)

Medium 1: Czapek-Dox medium.
Medium 2: Czapek-Dox medium with 0.4% sodium glutamate.
Medium 3: broth medium with 3% glucose.
Medium 4: broth medium with 3% glucose and 0.1% K_2HPO_4.
Medium 5: Czapek medium with 1% Katayama's peptone.

pH of this media was made 7.0 initially.

The reactions of media were found to change from initial pH. 7.0 to slight acidity and followed later by alkalinity, finally reaching a pH of 7.8 or even 8.0. According to results of my experiments the last one was the best of all for the production of antibiotic substance. This medium was distributed in 250 ml. amounts into 500 ml. flasks which were plugged with cotton, sterilized, inoculated with a suspension of spores of about 7 day's culture growth on Czapek-peptone-agar. The inhibitory power of the culture filtrate against staphylococci was assayed by dilution method mentioned above. It was revealed that a complete inhibition of staphylococci was shown in a 1:20 dilution after 3 days' incubation (pH. 6.4), in a 1:80 dilution after 4 days' incubation (pH. 6.7), in a 1:200 dilution after 6 days' incubation (pH. 7.0), in a 1:500 dilution after 7 days' incubation (pH. 7.1), in a 1:700 dilution after 9 days' incubation (pH. 7.2), in a 1:800 dilution after 10 days' incubation (pH. 7.3), in a 1:1200 dilution after 11 days' incubation (pH. 7.3), and thereafter the potency decreased slowly.

Just as it is true of " Penicillin " which was reported by Fleming, this

culture filtrate was highly active against most Gram-positive organisms, but not against most Gram-negative ones with the exception of gonococci, meningococci and some of the Gram-negative cocci found in nasal catarrhal conditions. *Streptococcus haemolyticus* was less affected than staphylococcus which was very sensitive, but was inhibited in a 1: 1,000 dilution. *Streptococcus viridans* was also very sensitive. *C. diphtheriae* was more strongly affected than staphylococcus, and was completely inhibited in a 1: 1,400 dilution. Pneumococci were inhibited in a 1: 400 dilution, gonococci in a 1: 300 dilution, and meningococci in a 1: 50 dilution. They appeared to be slightly less sensitive than other pyogenic cocci. *B. anthracis* which was the most sensitive, was inhibited in a 1: 2,000 dilution.

On the other hand, the members of the colon-typhoid-group were not affected as other similar intestinal bacilli such as *P. pyocyanea*, *Proteus vulgaris* and *V. cholerae*. It was also not effective against Friedländer's pneumobacillus, *Br. melitensis* and *B. dysenteriae Shiga*.

The Influence of the Kinds of Peptones (Myo-, Katayama-, Carno-, Uehara-, Kyokuto-, Poly- and Witte Peptone) upon the Inhibitory Power of the Culture Filtrate in Czapek-Peptone-medium

In reference to Czapek-peptone-media, kinds of peptones used had a great effect upon the potency of culture filtrate, but not upon the growth and the spore formation of this fungus. Now it was confirmed that the Czapek-medium with Katayama's peptone was superior to other media, while on the Czapek-medium with certain other peptones inhibitory powers of the culture filtrate against test organisms were found to be very low. Moreover I made a study concerning the culture media, which contained silk worm pupae extract, locust extract or sardine extract as the nitrogen sources. Although I could not find stronger inhibitory powers on the media containing these substances respectively, they may be useful as the nitrogen sources from industrial points of view.

The Rate of Killing of Staphylococci by the Culture Filtrate.

It possessed only a weak bactericidal property against some of the common pathogenic bacteria in comparison with a marked bacteriostatic one.

Inhibitory Power of Drop Fluid on the Surface of the Pellicle.

I collected the bright yellow drop fluid on the surface of the pellicle and examined its inhibitory power for staphylococci. They had a high

antibiotic titre. When the culture filtrate (pH. 6.4) in which the fungus was growing, inhibited staphylococci in a 1: 300 dilution, the drop fluid of this culture (pH. 6.8) tested at the same time inhibited completely the growth of staphylococci in a 1: 1,000 dilution.

Effect of Filtration.

Passage through a Seitz filter and a filter paper had no effect upon the antibiotic power, but through a Berkefeld filter diminished its power from a 1: 1,000 dilution to a 1: 400. Therefore, in order to obtaine a sterile active culture fluid, we generally used a Seitz filter.

Effect of Keeping at Refrigerator Temperature on the Anti-staphylococcal Power of the Culture Filtrate.

It was more stable if the reaction of the culture filtrate was kept from pH. 5.0 to pH. 7.0 and somewhat less stable at pH. 9.0, while considerably instable at pH. 2.0. The rate of this decrease of potency could be seen as follows:

Duration of Preservation	pH. 2.0	pH. 5.0	pH. 7.0	pH. 9.0
At time of filtration	600 –	600 –	600 –	600 –
after 1 day	100 –	600 –	600 –	600 –
after 3 day	100 –	600 –	600 –	600 –
after 5 day	100 –	600 –	600 –	600 –
after 7 days	50 –	600 –	600 –	600 –
after 9 days	50 –	600 –	600 –	600 –
after 11 days	50 +	600 –	600 –	600 –
after 13 days	50 +	600 –	600 –	600 –
after 15 days	50 +	400 –	400 –	400 –
after 17 days	50 +	400 –	400 –	400 –

The figures indicated the dilution titres of the filtrates at various pH.

Effect of Heat.

Heating for 30 or 60 minutes at 60°C or 100°C had no effect on antibiotic power of the culture filtrate, if it was neutral, very slightly acid or slightly alkaline, but if it was acid (pH. 2.0), its power was destroyed.

Effect of Concentration.

This antibiotic substance was thermostable at pH 7.0, so the culture

filtrate could be concentrated to its fifteenth volume by means of vacuum distillation at about 35°C and I confirmed that its antibiotic titre of the concentrated fluid became from 1: 400 to 1: 4,000 dilution titre.

Toxicity of the Culture-filtrate.

1 c.c. of the culture filtrate intraperitoneally or intravenously into a mouse weighing about 15 grm, induced no toxic symptoms, Also 30 c.c. injected intravenously into a rabbit showed no ill effect. 20 c.c. of sodium salt solution intramuscularly and subcutaneously into a rabbit weighing about 3,000 grm. produced no toxic signs.

Purification.

I) From 1,000 c.c. of this culture filtrate the active constituent was extracted at pH. 2.0 with ether. Then, the active principle contained in ether, was transferred into water as calcium or sodium salt. The last one was concentrated to gelatinous mass by means of vacuum distillation at about 30°C. Thus a brown powder was obtained when the gelatinous mass was allowed to evaporate to dryness in vacuo. This substance inhibited staphylococci in a 1: 200,000 dilution, if its filtrate was showing a titre of a 1: 800 dilution.

II) The antibiotic substance in the culture filtrate could be adsorbed by charcoal (added to 2–3%) at pH. 6.8 and then it was eluted into 70–80% acetone. After the pH of acetone solution was adjusted to 5.0–6.0 by adding the dilute HCl drop by drop, acetone was distilled off by means of vacuum distillation at about 30°C. in order to obtain about 20 c.c. of a brown water solution. From the latter solution the active principle was extracted at pH. 2.0 with ether and again transferred into water as calcium salt. After the procedure was repeated 3 times and the water solution was concentrated to 10 c.c. by means of vacuum distillation at about 30°C. a yellow powder is obtained when the final rich water was allowed to evaporate to dryness in vacuo at low temperature. This substance inhibited completely the growth of *Staphylococcus aureus* in a 1: 3,000,000 dilution.

Summary.

The fact that a substance which resembled very closely penicillin could be obtained from the culture filtrate of a strain of penicillia isolated by the author was revealed by the present paper. Thus it was shown that there was considerable evidence of biological similarity between penicillin and the isolated antibiotic substance.

1) A strain of penicillia which produced in cultures a powerful anti-

bacterial substance against Gram-positive bacteria was isolated from a contaminating blood plate in the laboratory.

2) The antibacterial power of the cultures reached its maximum in about 11 days at 20°–25°C. and after 12 days diminished, until it had almost disappeared in 27 days.

3) The best one of all culture media used for the production of the antibacterial substance was a Czapek medium with 1% Katayama's peptone (initial pH. was 7.0). In reference to the Czapek medium, kinds of peptones used had a great effect upon the potency of antibiotic substances, but not upon the growth and the spore formation of this fungus.

4) The culture filtrate inhibited the growth, in particular, of staphylococci, streptococci, *Bac. anthracis*, *Corynebact. diphtheriae*, gonococci, meningococci, and pneumococci, namely Gram-positive bacteria, but not the growth of *B. coli*, *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. enteritidis* *B. dysenteriae*, *Proteus vulgaris*, *P. pyocyanea*, or *Vibrio cholerae*, namely Gram-negative bacteria.

5) The rate of killing of staphylococci by the culture filtrate was very weak. It was thought, therefore, that its action was bacteriostatic rather than bactericidal.

6) The antibacterial titre of small bright yellow drops which were collected on the surface of the fungus was higher than the titre of the culture filtrate.

7) Passage through the Berkefeld filter diminished the antibacterial power, but not through the Seitz filter or filter paper.

8) At pH 2.0 the culture filtrate lost almost all powers after 1 day at the refrigerator temperature, but if its reaction was kept from pH 5.0 to 7.0, it could be preserved almost constantly after 14 days.

9) The active agent of the culture filtrate was thermostable, if it was kept from pH 5.0 to pH 7.0, but boiling at pH 2.0 for 30–60 minutes reduced the power considerably.

10) The culture filtrate was able to be concentrated to one fifteenth of its volume by means of vacuum distillation at about 35°C. without diminishing of the antibiotic titre.

11) The culture filtrate was non-toxic to animals in enormous doses.

12) The active constituent could be extracted as a calcium or sodium salt from the culture filtrate.

In conclusion, I wish to express my deep appreciation to Prof. M. Kuroya for much valuable advice and for examining this manuscript.

This work was conducted in the war time, from March to September, 1944. The fact described above is now a widely known knowledge, but in that time it was a newer contribution

to the research of chemotherapy.

M. Kuroya.

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Studies on Melanin.

VI. The Genesis of the Nevus Cell.

By

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Research on the genesis of the nevus cell in pigmented nevi is being carried on by many investigators. First came the Fibroblast theory (Simon, Rokitanski and Virchow), then the Bloodvessel-wallcell theory (Demieville), next the Lymphatic-vessel-endothelialcell theory (Recklinghausen) and the Chromatophore theory (Ribbert). These accentuate the mesenchymal origin. But when the Abtropfung theory was proposed by Unna, it was accepted by many investigators, especially on account of the dopa reaction of Bloch[1] that leads to the theory of the skin nerv origin of Masson[2] which is being much discussed.

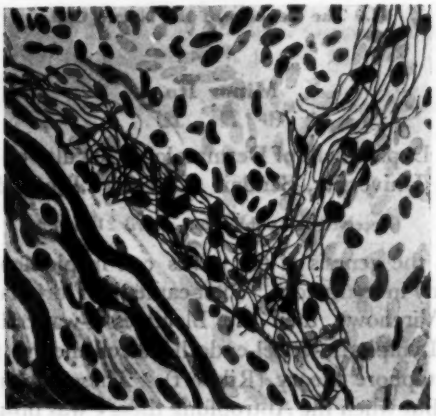
I have also made a study of the above subject. First of all, I concentrated on the examination of the relationship between the skin nerve and nevus cells by the advanced method of Bielschowsky's silver impregnation of neurofibril (Prof. Seto,[3] Tohoku University) and then discussed the problem of fiber formation of nevus cell by the Gitterfaser staining of Bielschowsky-Maresch.

Materials were taken from 13 patients and were fixed at least 10 months in neutral formalin (Seto's method). Sections about 30μ in thickness were made, stained and biopsied. At the same time, I studied the histology of neurofibroma, neurinoma, malignant melanoma, blue nevus and mongolian spot by the same method.

1. Microscopic studies of the Bielschowsky-Seto's neurofibril staining preparations. At nevus lesions the neurofibril bundles are prominent at the deeper layer of the derm under low power microscope, reminding one of the Polineurie of the French school. At places where nerve bundles penetrate or run close to nevus cell nest, their Schwann's cells are much increased in number. The nuclei of these Schwann's cells are so hypertrophied in a circuloid form that the nerve bundles seem to swell as a whole mixed with nevus cells, and consequently it is difficult to differentiate the nevus cell nucleus from that of the Schwann's cell either morphologically

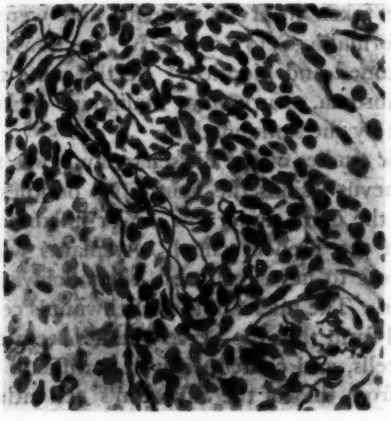
or dispositionally. The fine ramificated neurofibrils coil round the nerve bundle like a gabion, and their Schwann's nuclei give the impression of transition to nevus cells (see Fig. 1).

Fig. 1



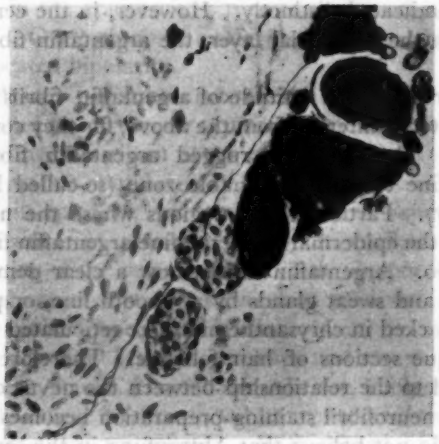
We can see more or less in all specimens neurofibrils running into the nevus cell nest of reticulated midlayer, and especially in several preparations the cross and oblique sections of nerve bundles are visible. The biopsy shows the particular figure of several looped and ramificated nerve bundles between nevus cells. The nuclei of Schwann's cells are typical long-oval and bend together with neurofibrils but some nuclei are so swollen that we can not distinguish them from nevus cells (Fig. 2).

Fig. 2



Several magnitudes of nevus cell nest and single nevus cell are situated sporadically and they come into contact with the course of one or two neurofibrils and fine vegetable nerve (Fig. 3).

Fig. 3



In several specimens of young children suggestive of early stage, nevus cells exist diffusely in the spongy tissue without the nest formation. The shapes of the cells are round, oval, comma-shaped or rarely stellate and some of them are connected with each other by their dendrites. We can see between them fine bundle of neurofibrils and nevus cell groups situated in the same course with neurofibrils. Sometimes the Schwann's cell and the nevus cell are twisted with each other and this fact shows the morphological transition between these two cells. In my observation of several specimens, I recognized the figure of something resembling endoneural apparatus, on which Masson based his nerve cell theory, i.e., morphlogy of Fibres naeviques, Faisceau neuroid, Lames foliacées, Corpuscules naeviques and Cellules claires in basal membrane.

The blood vessels are found near or in nevus cell nest but I have not been able to find any figure which shows the relationship between these vessel-wallcells and nevus cells or their transition in any part. The Abtropfung figure is refuted by the following observations of Gitterfaser staining slides.

2. Microscopic studies of the Bielschowsky-Maresch's Gitterfaser staining preparations. Results of observations are similar in all preparations, i.e., the nevus cell nests are completely packed with argentaffin fibrils which penetrate into the nests and show the fine reticulated ap-

pearance, and each nevus cell looks as if it is inlayed in each reticulum. Especially at some places where nevus cells are scarcely located, closely united figures of argentaffin fibrils and nevus cell protoplasma, and also the nevus cells, abundant with melanin, sticking to argentaffin fibrils botrioidally, are indicated distinctly. However, in the central part of big nevus cell nests in the superficial layer, the argentaffin fibril is not clearly stained out.

On the other hand, the attitude of argentaffin fibrils toward the epidermal layer is utterly different from the above, for they confront with each other decidedly. The minutely rugged argentaffin fibrils are located separately from fine clear non-stainable zone (so-called hyalinous membrane of Ranvier). Particularly at regions where the nevus cell nest is projected against the epidermal layer, the fine argentaffin fibrils draw a line between these two. Argentaffin fibrils show a clear demarcation against sebaceous glands and sweat glands by a smooth line, or pretty nevus cell nests which are packed in chrysanthemum-like reticulated picture observed around the oblique sections of hair follicule. Therefore, as the unclear point with regard to the relationship between the nevus cell and the epidermis in former neurofibril staining preparation becomes clear by Gitterfaser staining, I do not believe that Abtropfung in the sence of Unna can be upheld. Moreover, I have confirmed these observations by Mallory's staining.

Comment.

1. The relationship between the nevus cell and the skin nerve. In 1899, Soldan advocated that nevus cell belongs to a tumor of connective tissue cells originated from neurofibril sheath, but people took no notice of his opinion as just then the Abtropfung theory of Unna was becoming influential. However, in 1926 Masson[2] established the neural origin theory of nevus cells that was founded on the observations by his own method called Trichrome staining. According to him, the nevus cell comes from Cellules claires that corresponds to the Merkel-Ranvier's tactile cell in the epidermis. He pointed out the Fibres naeviques, Faisceau neuroide, Lames foliacées, Corpuscules naeviques in the derm and considered the nevus cell as a special form of skin nerve which has lost its sheath and axis-cylinder but has proliferated Schwann's cell. This excellent new theory called attention of the investigators. Chandler Foot[4] first supported it. Miescher[5] and Albertini made a supplemental experiment of Masson's method and they both helped to uphold the new theory. Then Feyrter,[6] suspecting its origin to be the endothelcell-like Häutchenzelle which is located between the external sheath of nerve, recognized this fact by a special myelinsheath staining. John[7] continued the study by the Biel-

schowsky-Gros's method and noticed the swelling and karyocytosis of Schwann's sheath as well as the abnormal course and ramification of neurofibril in nevus cell nest. He considered these as a manifestation of periferal nervous reaction which concerns the whole nerve tissue and noticed the Stalagmocyten in the epidermis, a type of proliferation and agreed to the bipolar theory. Ota[8] obtained analogous results to that of Masson, and therefore, advocated bipolarity.

I made clearer preparation by Seto's excellent modification. On account of the nature of the nerve tissue, I am unable to regard the abnormal figure of neurofibrils in nevus cell nests to be a simple incidental or passive transformation, but I am inclined to believe it has some active or causative relationship to the nevus cell formation. Especially in the Figure 1, I can see the process of transition of Schwann's cell of neurofibril to nevus cell by the twisting of the nerve bundles in the deeper layer like a gabion. I believe the above observation is the best evidence which support the theory of Schwann's cell origin of Masson.

2. The relationship between nevus cell and argentaffin fibrils. Demieville, Dalla Favera, Bauer, Löwenbach, Abesser, Ribbert, Oppenheim and others proved that there are fibrous substances around the nevus cells which penetrate into the nest reticulately. With regard to the origin of these substances, Dalla Favera, Takeuchi and others assumed that the nevus cell infiltrates into collagenic fibers, while Unna, Abesser, Fick and others considered the substances as a new born fiber from existing connective tissue. However, a school which maintains the mesenchymal origin of nevus cells (Recklinghausen, Bauer, Scheuber, Ribbert, Krompecher, Borst and Hausemann, Fischer and others) regards the fibrous substances as a product of nevus cells. But Kromeyer, together with Judalewitsch, suggested that the epidermal cell first drip down and then change into the connective tissue. He advocated this phenomenon as Desmoplasie. Masson advanced the theory that the fiber originated from the substratum of Schwann's sheath. Then Lucioni, Cohn, Chandler Foot, Feyrter and others recognized that the nevus cell is packed with reticulated fiber by several silver impregnations and advanced their own theories.

If we are to consider the argentaffin figrils as the Gitterfaser in a narrow sence, my observation leads us to call our attention to the reticuloendothelial tissue and it becomes favorable to the mesenchymal theory and conflicts with my own observation in the foregoing paragraph. But we can not consider the argentaffin fibrils to be always mesenchymal, especially, since Sekine[9] explicated recently in his embryo-morphological study that there exists neurogenic variety of argentaffin fibrils as well as mesenchymal and epithelial ones. So he recognized that the Neurilemmafaser is no other than the Schwann's sheath and that this argentaffin fibril is separ-

ated from the collagenic fiber of internal sheath and, therefore, held that it should undoubtedly belong to the neuro-ectodermal origin. On the other hand I identified the argentaffin fibril of the Schwann's cell on the Bielschowsky-Maresch's staining preparation of neurofibroma and neurinoma which I studied for the sake of consultation. It can be affirmed that the argentaffin fibril in my slides of nevus cell is the neurogenic fibril which originates from the Schwann's cell, and consequently, the theory of Soldan and Feyrter that maintains the origin to be the internal and external sheath of neurofibril can be refuted at the same time. But the parade-like arrangement such as seen in a neurinoma is scarcely found in nevus cells. This is easy to understand when we think of the difference between neurinoma which comes out in non-peripheral nerve bundle, and nevus cell which is a lesion at the periphery of skin nerves. The attitude of argentaffin fibrils on the epidermal layer is very clear as reported previously. Even at places where nevus cell nests come close to the epidermal layer, I have always maintained the presence of argentaffin fibrils. This fact corresponds to the presence of the so-called Randbindegewebe between nevus cell nest and epidermis which was noticed by Bauer[10] and traced by Demieville, Ribbert, Wieting and Hamdi, Soldan, Berblinger and others. I note such fact as a counter-evidence of Unna's Abtropfung theory.

3. The relationship between nevus cell and melanin. In accordance with the Abtropfung theory of Unna, the Dopa-oxydase theory of Bloch seems to be convincing of the ectodermal origin of nevus cells. But there is much doubt with regard to the theory, as Kissmeyer, Kaiserling and others have already pointed out. Moreover, the instance of dopa-positiveness in blue nevus and mongolian spot observed by Sato and Bahrawy of Bloch's school is not necessarily favorable to epidermal theory and I believe I can show the mistake of epidermal nature in my comparative study of blue nevus and mongolian spot, which I believe to be of mesenchymal nature.

Inclusion of melanin is not necessary in nevus cells, although abundance of melanin is generally found in the superficial nevus cells and it can also be found in the deeper cell nest or in scattered nevus cells. This fact is unexplainable as a photodynamic reaction and we are to find the new signification of dopa-reaction on this point. And I observed by Bielschowsky-Seto's staining preparation the vegetable neurofibril and its endoreticulum, particularly at parts where melanin is found in nevus cell nest or in scattered nevus cells. I also noticed such contact of vegetable neurofibril in the comparative study of malignant melanoma, blue nevus and mongolian spot. Therefore it is evident that there exists some relationship between vegetable nerve fibrils and melanin production. Namely, if a cell, be it a nevus cell of neuroectodermal origin or mesenchymal chromatophore (as it is the case with a mongolian spot), has some contact with the

termination of vegetable nerve fibrils, then the cell is possessed of melanogenic facility. Consequently, it may be assumed that melanin production is the result of the chemical transmission of vegetable nerve in the so-called Erfolgzelle, or shortly, a neurohormonal reaction (Vielter). Therefore it is proper to assume the participation of oxydase. The dopa-reaction, I conclude, is no other than a sign which reveals the secret of this process.

Summary.

1. By means of the Bielschowsky-Seto's method of neurofibril staining, the author have concluded that the nevus cell is not the production of the "Abtropfung" of epidermis (Unna), but originates from the neuroectodermal cell which is homogenetic with the Schwann's cell of Masson.

2. I have recognized the argentaffinity of the Schwann's cell by Bielschowsky-Maresch's staining, and have supported the neurogenic nature of nevus cell. But I am obliged to regect the so-called Abtropfung theory on account of the indifferent attitude of argentaffin fibril against the epidermis.

3. The author believes the melanin production depends entirely upon the chemical reaction in the cell which is in contact with the termination of peripheral vegetable nerve, and that it does not matter whether the cell is ectodermal or mesodermal.

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Hematopoietic Actions of Some Complex Salts of Cobalt.

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Since Waltner and Waltner it is well known that the cobalt salts produce polycythemias in various animals. The cobalt salts used so far are simple salts such as chloride or nitrate. I examined the hematopoietic effects of some of the complex salts of cobalt: (1) *Croceo salt*, (2) *Flavo salt*, (3) *Purpureo salt* and (4) *Carbonatotetraammincobaltinitrate*. Dosis is 3 to 4 mg as cobalt per kg of body weight per day, intravenously. Rabbits were used.

Croceo salt: This produced marked polycythemias, reticulocytosis and increase in hemoglobin content without affecting leucocyte counts, the effects are similar to those of ordinary cobalt salts.

Flavo salt: This complex salt, the isomer of Croceo salt, also produced polycythemias, but the action was far weaker than that of Croceo salt.

Purpureo salt: This salt, contrary to the above, induced no increase in red cell count as well as in hemoglobin concentration.

Carbonatotetraammincobaltinitrate: There occurred polycythemias, the effect being moderate in degree.

It is interesting that there were differences in the hematopoietic action among various complex salts of cobalt, even between the isomers.

My thanks are due to Prof. K. Matsuda whose suggestion as to the cobalt polycythemias made me carry out this study.

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Biochemical Studies on Carbohydrates.

CXXV. Glycidamins in Human Bile.

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Landwehr[1] regarded bile mucin as a mixture of globulin and bile acid, because the mucin (bull) he separated gave no reducing carbohydrate on acid hydrolysis and out of those components, on the other hand, a substance could be prepared which agreed with the mucin in composition, physical properties and reactions. Later on Paijkull[2] considered the mucin (bull) to be a nueleoalbumin on the basis that his preparations were free from bile acids but they contained phosphorus and gave, like nucleoalbumins, flocculent precipitates on pepsin-HCl digestion and further that the acid hydrolysate was uncapable of reduction. Brauer[3] put forward again another opinion. The canine bile mucin of this author was Molisch-positive and showed reducing force after acid hydrolysis and gave no precipitate on treatment with pepsin-hydrochloric acid, so that he announced it as a real mucin. Whether or not Brauer's substance involved phosphorus and bile acids is not reported.

More recently (1923) Logan applied several methods to purify the protein of ox bile and studied its decomposition products. He concluded that it is " a mixture of a comparatively large proportion of glucoprotein with a small amount of nucleoprotein." The conclusion came from the positive Molisch and Ehrlich reactions and low N content (13.5–13.8%) of the preparations, which gave also faint reactions of phosphorus and purine. However, the mere qualitative tests above can't establish the presence of " a large proportion of glucoprotein " and the N content of a protein will be also decreased by conjugation of lipids. Furthermore, he said that the acid hydrolysates had only slight reducing force or was perfectly devoid of this force.

Thus heretofore exceedingly divergent views have been expressed. By the aid of recent techniques the problem might be more accurately solved, so we took it up again employing human bile as a material. At first bile was collected from an A individual and after dialysis, acidulated for the

purpose of preparatory fractionation. And from each the deposit appearing thereby and mother liquid was obtained a crude protein (respectively named Mucin- and Mucoid-fraction). It was unable to make the proteins perfectly pure, but a mucin preparation free from phosphorus, though Gmelin- and Pettenkofer-positive, could be separated. The mucoid fraction was not particularly purified. It was Pettenkofer-negative and gave only faintly positive test for bilirubin but had more than a trace of phosphorus. Analysis disclosed both of the proteins to possess an abundant quantity of carbohydrate composed of acetylhexosamine, galactose and sulfuric acid in approximate molecular ratios of 3:5:1. The phosphorus-free mucin above was further treated with an alcohol-chloroform-sulfuric acid mixture according to Logan. Bile acid in it was diminished to a hardly detectable quantity but no remarkable change of hexosamine- and N-content caused.

In addition, the hexosamine in the bile mucin was identified by isolating it as pentabenzoate and hydrochloride from the hydrolysates of the protein. The mucin material used for this purpose was obtained from bile of a patient belonging to Group O. Nevertheless the kind of hexosamine involved is analogized (cf. Tiba[5]) to be one and the same irrespective whether the bile mucin displays A- or B- or O-group-activity.

In short the findings so far indicated the present mucin and probably also the mucoid to belong to the class of usual glycidamins.

As to the group-specifity, Nakamura[6] pointed to the anti-isohemoagglutinative potency of human bile mucin. Regretfully the substance which he called mucin was prepared only by evaporating bile by means of a fan, washing with alcohol and drying. The mucin in the present account and the polysaccharide, which was separated therefrom nearly in a pure state (s. Expermental), possessed some mentioned potency. The mucoid looked inactive in this connection.

Experimental.

Preparation Procedure of Bile Mucin and Mucoid.

Incipient fractionation of bile. Bile was collected from a patient (choledochus cancer) with fistula belonging to Group A. 4 *l.* of it were centrifuged and the green supernatant slightly turbid liquid (pH, 6.8) was dialyzed (in a cellophane bag) against running water for three days. Thymol was used as a preservative. The appearing precipitates having been rejected, the fluid was distilled to about 2 *l.* under diminished pressure, acidified to pH 3.8–4.0 with acetic acid (glacial and 30%), whereby again precipitation occurred, and after standing for one day, centrifuged. The centrifugate was washed with 96% alcohol several times and with ether

and dried in a vacuum desiccator—Mucin-fraction (20 gm.). The supernatant liquid became opalescent on two days' dialysis (pH rose to 4.6). The fine particles were changed into coarse flakes by addition of acetic acid (30%) to pH 4.0 of the solution and filtered off, and the transparent filtrate distilled in vacuo, eliminating half way some coming-out precipitate by centrifugation. The residue was dehydrated with alcohol and ether and placed in a vacuum desiccator—Mucoid-fraction, 1.8 gm.

Purification. 1. The mucin. The mucin fraction above was coloured quite green. For the purpose of decolorization, various organic solvents (glacial acetic acid, acetone, methanol, amylalcohol, ether, dioxane, ethyleneglycol monoethylether, chloroform, pyridine, benzene, toluene) were tested with combined use of acid and alkali. It was also tried to throw down the pigment with $CaCO_3$ from an alkaline (Na_2CO_3) solution by addition of lime water and passing of CO_2-gas. Since, however, none of the attempts suggested any successful means, only a simple procedure of dissolution in alkali with following precipitation by acidulation was resorted to and finally the product was made free from phosphorus by means of glacial acetic acid:—

15 gm. of the mucin fraction were kneaded at first with water and 2% sodium carbonate, and water increased up to the total volume of 640 cc. Most of the substance dissolved. The solution had a pH of 8.8–9.0. The insoluble part was centrifuged off and to the brown supernatant liquid 30% acetic acid added to pH 4.0–4.2. The precipitates were washed with 96% alcohol and ether and after drying in a vacuum desiccator, again subjected to the same operations. Subsequently the obtained powder was exhausted in a continuous extractor with ether for five hours and with acetone for four hours. The remaining substance weighed 9.9 gm., when dried. This was mixed with 10 cc. of water and 150 cc. of glacial acetic acid and shaken for three days on an apparatus. Centrifuged. The centrifugate was washed thoroughly with 96% alcohol and then with ether and dried in a desiccator by means of a jet pump. 7.2 gm. was the green product—Mucin (a).

It gave a viscous solution with alkalies (Na_2CO_3 and NaOH), but dissolved neither in water nor in acids (dilute acetic acid, 1% HCl). Biuret, Molisch and Ehrlich (not only the indirect but also the direct (weak)[7]) reactions were shown, whereas naphthoresorcinol reaction of hexuronic acid (Neuberg-Saneyoshi) was not. Hydrolysable sulfur was detected. Tests for bilirubin (Gmelin) and bile acid (Pettenkofer) resulted in positive, but those for phosphorus (by oxidation and following ammonium molybdate test) and cholesterol (Liebermann-Burchard) in negative. Regarding quantitative analyses s. below.

0.8 gm. of this substance was dissolved in 100 cc. of water by the aid

of 5% Na_2CO_3 (pH of the obtained solution, 8.8) and, to remove bile acids, treated twice with 75 cc. of alcohol, 25 cc. of chloroform and 2.6 cc. of H_2SO_4 according to Logan. The material was then washed with 96% alcohol and dried in a vacuum desiccator—Mucin (b). Yield, 0.18 gm. Although bilirubin was definitely present in it, bile acid was scarcely found.

2. *The mucoid.* 0.6 gm. of the mucoid fraction was taken up in 20 cc. of water and after centrifuging off of a small insoluble part, acidified with 30% acetic acid to pH 4.0 and the appearing precipitates again rejected. The supernatant liquid was dialyzed for three days and distilled in vacuo to about 1 cc. and five volumes of 96% alcohol added. And the deposit was washed with alcohol and ether and dried in a vacuum desiccator (H_2SO_4). A product (0.3 gm.) with greenish hue was yielded. It was transparently soluble in water as well as in dilute acid. Biuret, Molisch and both the direct (weak) and indirect Ehrlich (Osaki-Turumi procedure) reactions were given. Neuberg-Saneyoshi and Pettenkofer, negative. Gmelin, slightly positive. No small amount of phosphorus was detected.

Quantitative Analysis of the Products above.

Their analytical figures obtained by our usual methods are seen in Table I.

TABLE I.

Composition of Mucin from Human A Bile, its Polysaccharide Separated and Mucoid from the Same Source.

Analysis	Mucin untreated with Logan mixture (a)		Mucin tr. w. L. mixt. (b)	Carbohydrate sep. fr. Mucin (a)		Mucoid	
	In per cent	In equivalents p. eq. wt.	In per cent	In per cent	In equivalents p. eq. wt.	In per cent	In equivalents p. eq. wt.
N	10.4		10.3	3.6	1.59	9.4	
Hexosamine	14.0	1.0	14.5	29.0	1.0	13.1	1.0
Galactose	23.8	1.69		50.0	1.72	24.0	1.80
Indole r.	24.0			50.6		24.4	
Orcinol r.	23.5			49.4		23.0	
Diphenyl-amine r. I	24.0			50.1		24.6	
Hydrolysable sulfer	0.79	0.32		1.72	0.33	0.56	0.24
Iodine use as glucose				5.9			
Ash	1.7		3.1	3.4		2.1	

Separation of the Polysaccharide from the Mucin.

A lot of the mucin untreated with Logan mixture (above) was hydrolyz-

ed with alkali and then glycol-soluble fragments collected. These were fractioned by means of glacial acetic acid, but only a bilirubin-stained preparation was given. Hence, in the next, the glycol-soluble substances from a second lot of the mucin were dissolved and after addition of $CuSO_4$, precipitated together with cupric hydroxide by means of alkali. The final product here was fairly satisfactory, and hence the latter trial will be described in full:—

3 gm. of the mentioned mucin were dissolved in 90 cc. of 1.5 n. NaOH and stood for two days in air (room temp., 3°–8°C). After addition of 30% acetic acid to pH 4.2 and centrifuging off of the precipitates, the supernatant liquid was dialyzed for three days against running water, whereby precipitation happened anew. The deposit having been discarded, the fluid (pH, 4.6) was then evaporated by vacuum distillation. The remaining syrup was dehydrated with 95% alcohol and washed with alcohol and dried. The powder given was kneaded to a dough with a little volume of glycol, and after increasing the solvent to 15 cc., agitated. Then the insoluble part was centrifuged off and the brownish clear supernatant fluid precipitated with 80 cc. of 96% alcohol. The precipitates were washed three times with alcohol and dried. 300 mgm. of substance were secured, which gave the biuret reaction though faint.

The product was taken up in 10 cc. of water, removed from a small part undissolved and 2 cc. of 1/2 m. $CuSO_4$ added. The mixture was made strongly alkaline (pH>10) by addition drop by drop of 10% NaOH, and the precipitates separated by centrifugation. To the supernatant liquid were again added 2 cc. of the $CuSO_4$ solution and alkali and again the precipitates centrifuged. These operations were repeated more three times. The centrifugates were united and washed with dilute alkali of pH>10 several times (by agitation and centrifugation), until a renewed washing remained uncoloured. Thereafter they were suspended in 12 cc. of water and 10% HCl added in small portions to dissolve them (pH of the solution was below 2.0). Some insoluble substance was removed and the solution precipitated with 70 cc. of absolute alcohol. The deposit was washed once with 20 cc. of absolute alcohol and three times with 20 cc. of absolute alcohol containing a few drops of 10% HCl, adding a small amount of anhydrous sodium acetate when a turbid washing was given on centrifugation. When the substance was thus freed from the copper stain and became perfectly white, it was washed with absolute alcohol and ether and dried—120 mgm. On the other hand, 2 cc. of the $CuSO_4$ solution were added to the united alkaline washings (violet-tinged) of the centrifugates, which consisted of $Cu(OH)_2$ and the carbohydrate, above. The mixture was distinctly alkalized and the centri-

fugate dissolved with HCl as described. Again the solution was alkalized and centrifuged. But the supernatant liquid[8] here was not clear, so the centrifugate was roiled after addition of sodium sulfate and centrifuged over again. This centrifugate was further subjected repeatedly to dissolution by the aid of HCl and precipitation by the aid of NaOH, until a non-colored alkaline supernatant liquid was obtained. The final centrifugate was dissolved in a similar manner, the acid solution precipitated with alcohol and the deposit washed with HCl-containing alcohol, plain absolute alcohol and ether in sequence and dried. 78 mgm. of white powder were yielded.

The both products gave a clear solution in water (pH, 4.4–4.6) and agreed perfectly in following reactions: Biuret, ninhydrin, Pettenkofer, Gmelin—negative; Molisch (violet), Osaki-Turumi (the direct faint; the indirect distinct)—positive. Besides, they gave the positive Sakaguchi, modified Millon (mercuric sulfate in sulfuric acid and sodium nitrite were used) (very weak) and Ehrlich diazo (very weak orange coloration) reactions.

Quantitative figures of the first product are listed in Table I. The same substance had the rotation in water:

$$[\alpha]_D^8 = \frac{-0.42 \times 2.9939}{0.0218 \times 1.0050 \times 1} = -57.4°.$$

Nature of the Hexosamine in Bile Mucin.

To establish the nature of hexosamine in the mucin, bile was collected afresh from a Group O individual with fistula after choledocholithotomy. Isolation of the mucin was executed in a like way as before and from 3700 cc. of the bile 19.3 gm. of a preparation (untreated with the alcohol-chloroform-H_2SO_4 mixture of Logan) were obtained. From its hydrolysates the hexosamine was isolated as pentabenzoate and hydrochloride.

1) Identification of the amino sugar as a benzoate.

5 gm. lots of the mucin were treated with 1.5 n. NaOH, and 500 mgm. of the glycol-soluble fragments were separated by the processes as noted (s. the section concerning the separation of the polysaccharide from the mucin). The fraction was heated in a boiling water-bath with 15 cc. of 5.5 n. HCl under reflux for four hours. The dark sediment that appeared was discarded by centrifugation and the supernatant liquid transferred to an evaporating dish and condensed on a water-bath to a syrup. Then this was taken up in 10 cc. of water and filtered and the filtrate condensed in a similar manner. The operations were repeated once more. And the evaporated residue was dissolved again in 10 cc. of water, an equal volume

of the saturated lead acetate mixed, and H_2S gas passed after centrifuging off of the precipitates. The filtrate of lead sulfide was distilled at a low temperature to 6 cc. with following elimination of acetic acid with ether (treated in a continuous extractor for four hours). Finally it was shaken vigorously with 9 cc. of 10% NaOH and 1 cc. of benzoylchloride under cooling with ice. Mucilaginous precipitates which separated were washed with 0.1 n. NaOH and water and fractioned in the usual way with alcohol and chloroform, and glucosamine pentabenzoate was obtained in a crystalline form from methanol (The methanol solution was decolorized by means of charcoal). The crop of the crude crystals (F.P. 204°C) amounted to 56 mgm. By two recrystallizations from methanol the melting point was raised to 214°–215°C and by the third no more elevation effected.

Rotation in pyridine:

$$[\alpha]_D^{20} = \frac{0.26 \times 2.1115}{0.0123 \times 0.9810 \times 1} = +45.5°.$$

N (micro Kjeldahl), 2.06% (calc., 2.00%).

2) Identification of the amino sugar as hydrochloride.

Hydrochloride of the hexosamine in question was separated by the method which Hiyama[9] adapted to a frog mucin:—

12 gm. of the same mucin material as in *1)* were immediately heated with 200 cc. of 5.5 n. HCl involving 4 gm. of stannous chloride. The hydrolysate was filtered and the filtrate bubbled with H_2S-gas. The centrifuged supernatant liquid was distilled in vacuo to about 10 cc. and extracted thoroughly with butanol in a Dakin's apparatus for ten hours. The syrupy residue was diluted with 20 cc. of water and the insoluble part rejected, then the solution was distilled similar to above and to the remaining thick solution were added 10 cc. of absolute alcohol. Some precipitate that came out was filtered off and the filtrate placed in an ice-chest after addition of 1 cc. of acetone. Crystals appeared in small plates which were brought onto a suction funnel—90 mgm. These were taken up in 2 cc. of water and the solution removed of a small quantity of insoluble contaminants evaporated to syrupy consistency and stood after addition of 5 cc. of 96% alcohol and 0.5 cc. of acetone. Deposits were again recrystallized in a like manner. The product amounting to 43 mgm. had the same appearance as on first crystallization. On heating it began charring at about 180° and did not melt even at 290°C. Optical rotation in water: +88.7° after 7 minutes from dissolution and +71.9° ($[\alpha]_D^{15} = \frac{0.54 \times 3.1529}{0.0236 \times 1.002 \times 1}$) at equilibrium (after 4½ hours from dissolution). N (micro Kjeldahl), 6.48% (calc., 6.48%).

Test for Anti-isohemoagglutinative Activity.

The procedure was the one hitherto employed in our Institute. Both the mucin from the bile of an A individual (untreated with Logan mixture) and its polysaccharide separated by the alkali-copper method (above) inhibited specifically the isoagglutination of A erythrocytes at a dilution of 1:1,000–2,000, but the mucoid from the same source only at a dilution of 1:50–100. The potency of the mucoid is too low to accept as the own property of the substance. Both A and B sera used had agglutinin titre $\times 160$.

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Biochemical Studies on Carbohydrates.

CXXVI. Structural Study of A-specific Carbohydrate from Mucus Mucin of Pig Stomach.

Second Paper.

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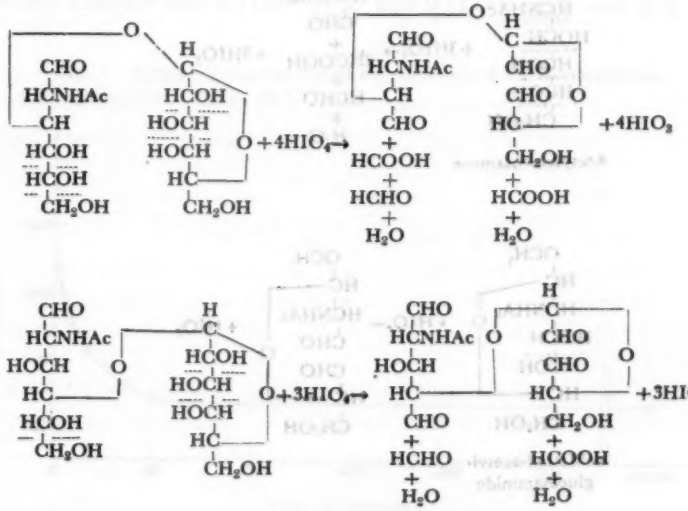
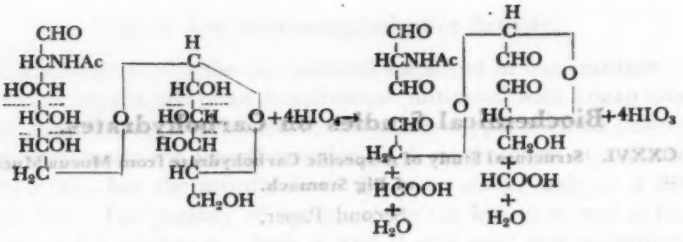
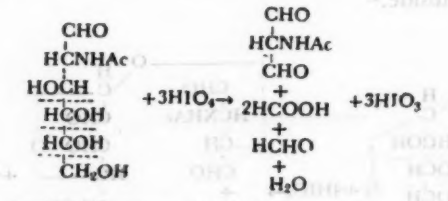
The unit disaccharide of the carbohydrate in the heading could be identified with acetylglucosamine(1,5)-4-β-*d*-galactoside(1,5) from its use of periodic acid and enzymatic cleavability and by its synthesis. This account deals with the experiments concerned except the synthesis. That the unit disaccharide is a galactoside but not a glucosaminide has been already confirmed in the foregoing investigation.[1] Consequently, 4 or 3 or 4 mols of periodic acid are to be reduced according as the disaccharide is 3- or 4- or 6-galactoside.[2]



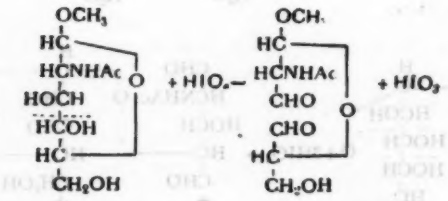


In fact, the disaccharide in question reduced 3 mols of the acid per mol, hence it is acetylglucosamine(1,5)-4-galactoside(1,5). Although, in the above reasoning, the ordinary pyranose configuration was presumed for both of the monosaccharide components, furanose form of galactose causes no divergency in the periodic acid consumption, and acetylglucosamine(1,4)-5-galactoside uses one and the same quantity of the acid as acetylglucosamine(1,5)-4-galactoside. But these possible structures have been excluded by synthesis, as will be informed of in the third paper.[3]

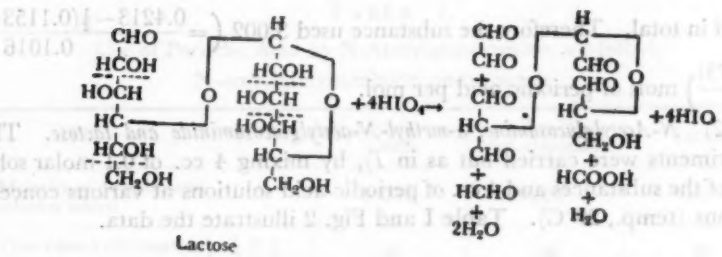
As controls acetylglucosamine, *α*-methyl-acetylglucosaminide and lactose were tested and found to use respectively 3, 1 and 4 mols of periodic acid per mol, coinciding with theory.



Acetylglucosamine



α-Methyl-acetyl-glucosaminide



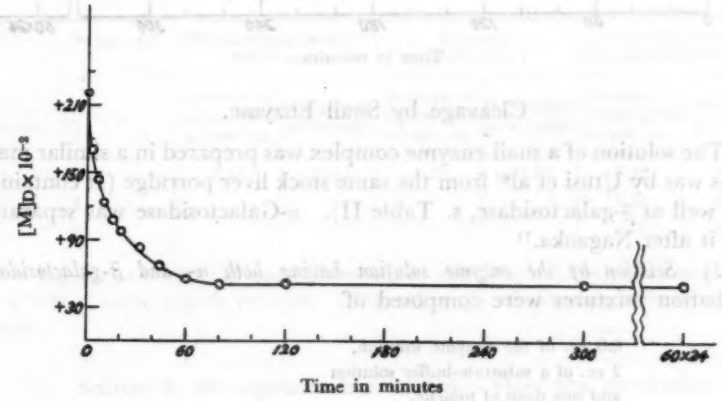
Furthermore, a snail enzyme solution involving α- and β-galactosidase cleft the unit disaccharide, but α-galactosidase separated therefrom did not. This finding indicated that the substance is a β-galactoside.

Experimental.

Periodic Acid Consumption.

1) The unit disaccharide (*N-acetylglucosamine-galactoside*). To 5 cc. of 0.1016 molar solution of this sugar, whose rotation had arrived at equilibrium, was added an equal volume of 0.4213 molar HIO_4[4] solution and stood at 20°C. The change of rotation (D-light) ceased after 80 minutes from the commencement of reaction (Fig. 1). At the 80th and 90th minute, 2 cc. of the mixture were taken out to estimate periodic acid remaining unreduced by the arsenious oxide method of Fleury and Lange,[5] and $0.1153\times\frac{5}{1000}$ and $0.1173\times\frac{5}{1000}$ mol of it were

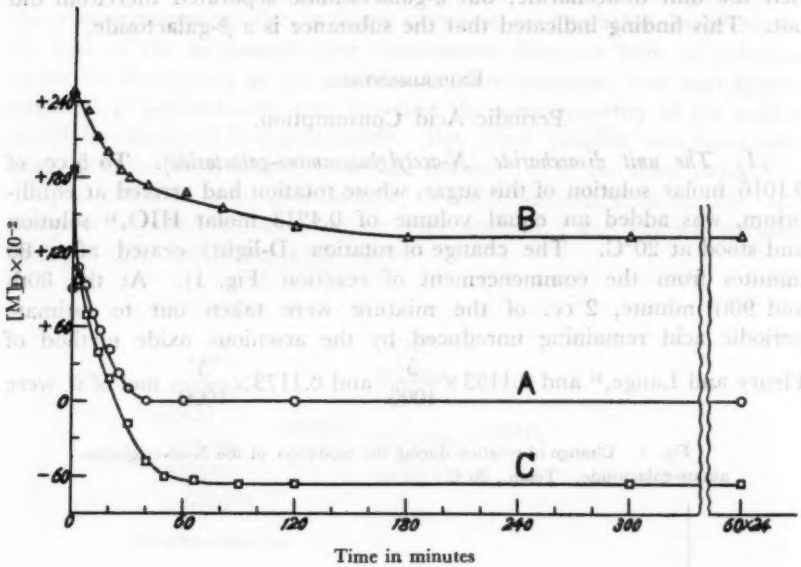
Fig. 1. Change of rotation during the oxidation of the N-acetylglucosamine-galactoside. Temp., 20°C



found in total. Therefore the substance used 3.002 $\left(=\frac{0.4213-\frac{1}{2}(0.1153+0.1173)}{0.1016}\right)$ mols of periodic acid per mol.

2) N-Acetylglucosamine, α-methyl-N-acetylglucosaminide and lactose. The experiments were carried out as in *1)*, by mixing 4 cc. of 0.1 molar solution of the substances and 4 cc. of periodic acid solutions at various concentrations (temp., 20°C). Table I and Fig. 2 illustrate the data.

Fig. 2. Change of rotation during the oxidation of N-acetylglucosamine (A), α-methyl-acetylglucosaminide (B) and lactose (C). Temp. 20°C.



Cleavage by Snail Enzyme.

The solution of a snail enzyme complex was prepared in a similar manner as was by Utusi et al[6] from the same stock liver porridge (It contained α- as well as β-galactosidase, s. Table II). α-Galactosidase was separated from it after Nagaoka.[7]

1) Scission by the enzyme solution having both α- and β-galactosidase. Incubation mixtures were composed of

0.6 cc. of the enzyme solution,
2 cc. of a substrate-buffer solution
and one drop of toluene,

TABLE I.

Use of Periodic Acid by N-Acetylglucosamine, α-Methyl-N-acetylglucosaminide and Lactose.

Substance	N-Acetylglucosamine		α-Methyl-N-acetylglucosaminide		Lactose	
Molarity of periodic acid solution added	0.4213		0.2202		0.5219	
Time (min.) till cessation of rotation change (after commencement of reaction)	40		180		90	
Time (min.) of measuring the excess periodic acid (after commencement of reaction)	40	60	180	300	90	120
Mols of the excess periodic acid	0.1208	0.1089	0.1193	0.1189	0.1143	0.1269
(Average)	(0.1149)		(0.1191)		(0.1206)	
Mols of periodic acid consumed per mol	3.065		1.011		4.013	

and immediately before and at intervals during incubation (37°C), 0.4 cc. was taken out to determine the iodine use by Macleod-Robison method.[6] The unit disaccharide was cleft as is seen in Table II, where the control runs with phenol-α-*d*-galactoside and lactose are also illustrated. In conformity with the findings of Nagaoka,[7] buffer solutions of different pH's were used in the tests of phenol-α-galactoside and of lactose.

TABLE II.

Scission of Sugars by the Snail Enzyme Complex Involving α- and β-Galactosidase.

Substr. sol. / Time of incub. (h)	Phenol-α-galactoside*† (0.039 mol.)		Lactose‡ (0.041 mol.)		Acetylglucosamine-galactoside (0.039 mol.)*	
	Increase of iodine (0.02 n.) use cc.	Hydrolysis percentage	Increase of iodine (0.02 n.) use cc.	Hydrolysis percentage	Increase of iodine (0.02 n.) use cc.	Hydrolysis percentage
1	2.13	19.21	0.25	21.01	0.12	10.08
3					0.22	18.49
9					0.38	31.93

* Digested at pH 3.2 (Michaelis buffer solution employed). † Free phenol reduces 9.44 cc. of 0.02 n. iodine solution per mgm. ‡ Digested at pH 2.5 (Sörensen buffer solution employed).

2) *Scission by the α-galactosidase solution.* Here the incubation mix-

tures were made up of

1 cc. of the enzyme solution,
0.5 cc. of a substrate-buffer solution
and one drop of toluene.

Temp., 37°C. Increase of iodine use was estimated on 0.4 cc. of the mixtures in a like way as before. Table III illustrates the results.

TABLE III.

Scission of Sugars by α-Galactosidase of Snail Liver.

Substr. sol. / Time of incub. (h) / pH*	Phenol-α-galactoside (0.078 mol.)		Lactose (0.082 mol.)				Acetylglucosamine-galactoside (0.078 mol.)			
	Increase of iodine (0.02 n.) use cc.	Hydrolysis percentage	Increase of iodine (0.02 n.) use cc.				Increase of iodine (0.02 n.) use cc.			
	3.2		2.2	3.2	4.5	6.2	2.2	3.2	4.5	6.2
3	1.02	10.61		0						
9	2.25	23.41	0	0	0	0	0	0	0	0

* Adjusted by Michaelis buffer solutions.

SUMMARY.

1) The unit disaccharide (N-acetylglucosamine-galactoside) of the A group carbohydrate (separated from mucus-mucin of pig stomach) reduces 3 mols of periodic acid per mol.

2) It is cleft by β-galactosidase from snail liver.

3) From these findings it is assumed that, in this compound, galactose links position 4 of acetylglucosamine through β-glucosidic union, if glucosamine takes the pyranose form.

4) N-acetylglucosamine, α-methyl-N-acetylglucosaminide and lactose reduce 3, 1 and 4 mols of periodic acid respectively per mol.

Through the Grant Committee of National Research Council was given a grant from the Education Department which enabled us to carry out this work.

H. M.

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3) Yosizawa, Tohoku J. Exp. Med., in press.

4) Prepared according to Jackson and Hudson (J. Am. Chem. Soc., 1937, **59**, 998).

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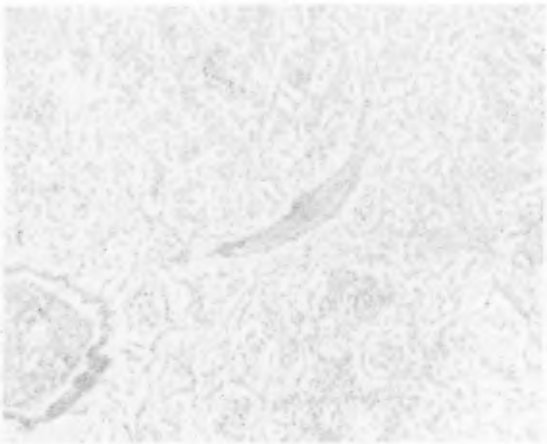
7) Nagaoka, ibid., 1949, **51**, 137.
8) Macleod and Robison, Bioch. J., 1929, **23**, 517.



The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

Contribution to the Study of Argyrophilic Fibers.

Preliminary Report: "On Angioreticulin"

By

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(Received for publication, February 14, 1950)

Of late, researches on the argyrophilic fiber (reticulin) aroused much interest among pathologists of this country. As there is no absolute standard of silver impregnation methods, it would, therefore, be reasonable to differentiate black stained fibrils in accordance with their milieu and the impregnation methods applied, under careful examination of otherwise stained specimens at the same time. By means of Bielschowsky-Foot or Perdrau's technique, there comes into notice, in the brain, the vascular pattern, presenting a fibrous bridge between capillary loops (cordon junitiv Cajal) occasionally. Turning to the more highly vascularized nervous tissue e.g. various forms of glioma, many suggestions on the hyperplastic change of the blood vessel were obtained. The figure shows an oligodendroglioma of the brain impregnated by Foot' modification (paraffin section). The writer intends to propose a name, angioplastic reticulin (angioreticulin in short) for the silver fibril supporting the capillary tube universally.

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Studies on Melanin.

VII. Mongolian Spot Cell and Blue Nevus Cell.

By

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(Received for publication, April 22, 1949)

In contrast with black or brown color tone of pigmented nevi, there are two kinds of congenital deep blue pigmented spots: mongolian spot and blue nevus. The former appears generally on sacral region of newborn and fades within several years, while the latter appears anywhere but is comparatively seldom and exists for life. These two nevi resemble each other in the point that they are both due to the melanophore of deeper dermal layer. Their difference and entity have also been studied at various times, although we have not come to a definite conclusion as yet.

I[1] studied formerly the so-called nevus cell of pigmented nevi and described the theory of neuroectodermal origin. In this article I intend to investigate the genesis of mongolian spot and blue nevus by means of methods similar to that referred to in my former article: principally Bielschowsky-Seto's neurofibril staining and Bielschowsky-Maresch's Gitterfaser staining were used.

Results of Investigations.

1. Mongolian spot. The results of investigations of the sections taken from 11 cases of fetal six to three months corpses and one case of two months living body, are similar to the description given by my predecessors. In other words, I indicated the mongolian spot cell in lower half layer of derma as fusiform, oval, stellate or somewhat elongated string-like melanophores lying along the paths of connective tissue and with their pigment granules greater and denser than those of the basal layer of epidermis. They are so filled with pigment granules that we can hardly distinguish their nuclei. These pigment granules do not fade by hydrogen peroxide procedure. In my examination of peroxydase in a section taken from living body by Okano-C method, I found peroxydase positive granules among the black granules of melanin. This suggests that there is some relation-

ship between this and the positive data of dopa-reaction by Bloch-Bahrawy. Then I examined closely the arrangement of these chromatophores and found that although most of them exist parallel to cutaneous surface, not all coinside with the paths of connective tissues, but some come in contact with small blood vessels; some are joint with their cellular processes; some twist with blood vessels like a gabion. We can often see chromatophores near small bundle of neurofibril and a little more of them at places where blood vessel and neurofibril cross each other. But the conglomeration or proliferation of chromatophores with abnormal ramification and winding of cutaneous nerve fiber as its center, such as I found in my previous report on nevus cell, can not be seen anywhere. Moreover, no figure suggesting transition to Schwann's cell or nothing like an endoneural apparatus (Fibres naeviques, Lames foliacées) specially mentioned by Masson, is seen. The mongolian spot cells exist next to ductus sudoriferus or can be seen around arrector muscle but are rarely seen near sebaceous gland and hair follicle.

On the Gitterfaser staining slides, I found the above mentioned figure of chromatophores around blood vessel packed within argentaffin fibril and another figure closely connected to argentaffin fibril in subcutaneous adipose tissue, but with regards to mongolian spot cells in connective tissue I could not ascertain its relationship to argentaffin fibril. Only in preparation of six months fetus, big stellate or oval chromatophores are found plentifully in the part where collagenic fiber is rare and argentaffin fiber abundant. I could not discover in every case the inlaying figure of each cell into each reticulum, which means that the mongolian spot cell may have some partial relationship to reticulum tissue and may be partly connected with the so-called precollagenic argentaffin fibril in its embryonal stage.

2. Blue nevus. The first case, 18 years old female, has light blue rice corn sized nevus on her forearm; the second case, 64 years old male, has indigo blue horse bean sized nevus on his hairy head; and the third case, 21 years old female has deep blue nevus on her buttock. Results of examinations of these cases are as follows: the first case has a small amount of chromatophores scattered horizontally in connecive tissue below the middle layer of the derma, and in contact with blood vessels there are some in fusiform and stellate. Therefore, we can not distinguish them from mongolian spot cells. However, latter two cases are abundant with chromatophores and this coinsides with the description of Tièche,[2] Sato[3] and Imschentzky.[4] These chromatophores run together with collagenic fiber in the range from the middle layer of derma to the deeper layer and they look like a bundle as a whole located horizontally in stratum reticulare and obliquely or vertically in subcutaneous adipose tissue. In case their black granules are dense, the neighbouring parts look like black fiber, and in case they are scarce, we can see their nuclei in their centers. These

chromatophores are found coincidentally in or among diffused collagenic fibers. Most part of these collagenic fibers show purplish red metachromasia by Mallory's staining. This may be presumably a sort of degeneration of collagenic fibers induced by the specific biological status of tissues containing chromatophores. There are stellate, fusiform, comma-shaped and, rarely, ovoidal chromatophores among or near fibrous chromatophore bundle (above mentioned) and their black granules are rather large. But chromatophores which have small granules show oval nuclei. Moreover, I found in my preparations small amount of large stellate inmature form which had been found by Imschentzky in derma of monkey but not in blue nevus. Stellate and fusiform chromatophores are shown in contact with blood vessel or ductus sudoriferus and sweat gland. The elastic fiber disappears almost completely in parts where fibriform chromatophores are densely located and at the layer above them.

The relationship between neurofibril bundles and melanophores studied by Bielschowsky-Seto's staining is quite different from that of nevus cells. The clearly demarcated cross-sections of neurofibrils (N) are indicated among groups of fibrous melanophores and there is no figure suggesting affinity between neurofibrils and melanophores. Moreover, that neurofibril is slightly lighter stained and has less amount of Schwann's nuclei than other neurofibril found apart from chromatophore nest. These characteristics are suggestive of embryonal unripe neurofibril (Fig. 1).

By Bielschowsky-Maresch's staining in part where fibrous chromatophores are densely located we can hardly distinguish Gitterfaser, but ar-

Fig. 1

gentaffin fibril (stained fresh blue by Mallory's method) is easily distinguished in parts where stellate chromatophores gather together. But all of these cells are not packed within argentaffin reticulum. The stellate processes of some of these cells look like an argentaffin fibril because of these dense argentaffin granules. In the circumference of small blood vessels, however, melanophores are packed distinctly in reticulum tissue. The clearly demarcated neurofibril among melanophore groups is lighter stained and the auther believes this to be an embryonic unripe neurofibril according to the results of previous neurofibril staining. However such belief is not conclusive in view of the labil character of silver impregnation method.

The biochemical characteristics of the pigment contained in chromatophores are as follows: ferrous reaction is negative, silver reaction positive, therefore it is assumed to belong to melanin. Faints slightly with hydrogen peroxide, especially the pigment in stellate type chromatophores has stronger resistensy than that of fibrous type one and remain unchanged even by hydrogen peroxide procedure of one week duration (daily exchange of hydrogen peroxide). Thus according to the above mentioned characteristics and the fact of the simultaneous existence of positive and negative cells against dopa reaction (Sato and Stranz), it seems that there are several stages of chromatophores of blue nevus with regards to the morphological and biochemical character and especially in connection with the attitude of oxidation-reduction. Kreibich[5] reported the existence of lipoid in 1 case of blue nevus which, however, I was unable to confirm by sudan III staining.

Summary of aforesaid observation is as follows: in the comparative study of the characteristics of melanophores between mongolian spot and blue nevus, I recognized exact homology concerning the morphlogy, location, disposition and biochemical attitudes (non-faintness with hydrogen peroxide and positive dopa reaction) and especially the relationship between neurofibril and chromatophore which I examined very closely, is quite different from that of nevus cells (previously reported). There is no affinity between chromatophore and neurofibril. On account of this definite distinction, mongolian spot and blue nevus coinside and both of them show pigment cell nests which are partly packed within reticulum tissue or diffused nests against argentaffin fibril. Yamamoto[6] reported that as he found no transition between these two pathological hyperpigmentations he could not decide definitely that they were of the same species. But the first case of my blue nevus preparation can be considered to be the evidence of histological transition and, therefore, I believe these two pathological hyperpigmentations are essentially due to the almost homogenetic chromatophores.

Comment.

About the origin of the mongolian spot cell, Bälz visualized the epidermal origin but the mesenchymal theory of Adachi was universally accepted. Kawamura, Kiyono, Katsunuma and others advocated the histiocytic theory and surmised that this is organically homologous but functionally different from the so-called chromatophores. Especially the cutaneous pigment of the monkey was minutely studied comparative-anatomically by Adachi and more recently by Imschentzky. They indicated the existence of dermal melanoblast in deeper layer against superficial so-called chromatophore, and emphasized that this is of the same species to which mongolian spot cell belongs. The difference between these two is merely that the pigment of the monkey is reciprocal proportionally in epidermis and derma. In other words, when it is abundant in epidermis, it is scarce in derma, while mongolian spot (dermal pigment) is prominent in colored races (epidermal pigment). The origin of chromatophores of blue nevus has been believed to be chiefly mesenchymal since the publication of Tièche. Imschentzky studied five cases of blue nevus and found it difficult to distinguish them from dermal melanoblasts of monkey. Although he could not notice in blue nevus the big stellate cell (unripe form) such as seen among monkeys, he made an interpretation that dermal melanoblast may be treated as a system sui generis.

As stated above, according to my observation, mongolian spot cell and blue nevus cell belong to the same species and they are different from nevus cell (reported previously) and, therefore, I definitely reject the ectodermal origin, and believe that is chromatophore of dermal origin as mentioned by Adachi and Imschentzky.

However, the problem, namely, from what kind of mesenchymal tissue the said chromatophore comes, can hardly be surmised. I also noticed in my observation of Bielschowsky-Maresch's staining, a view which suggests its relationship to reticulum tissue and in part unseparably mixed figure of chromatophore and argentaffin fibrils, thus I can not conclude hastely that such chromatophore originates indiscriminately from histiocyte or cell of reticulum tissue. What's more, silver impregnation belongs to an artificial procedure as in both neurofibril and Gitterfaser stainings and its labil character is universally known. Therefore, the entity of argentaffin fibril is still disputable, and on the other hand the extracellular protoplasma theory of Hueck, the tissue culture of Maximow, the biochemical experiment of Nageotte and other new knowledges appear one after another. In Japan, Sekine studied this recentely, thus I think we can expect further development in this problem. The auther refer only to the abnormality of congenital factor on one hand, and to the special biochemical status of

the foci on the other: the former, indicated by the co-existence of chromatophores and argentaffin fibrils, leads to the assumption of the remainder of so-called precollagenic argentaffin fibrils, while the latter are shown by the metachromasia of collagenic fibers in region abundant in chromatophores. So I want to reserve the problem as to its origin for the time being.

I can not overlook following facts: that Stranz[7] observed contemporal existence of the so-called nevus cell in 3 out of 11 cases of blue nevus; that similar mixed existence of nevus cell was observed in 1 case each by Sato, Armuzzi, Dubreuilh[8] and Petges. I think that foregoing cases are the associated cases of blue nevus and pigmented soft nevus because of the frequent instances of associated symptoms in many congenital abnormalities and the fact of the participation of both ectoderma and mesoderma observed in cases of phacomatosis. And I do not think this can be regarded as the data of epidermal theory of blue nevus.

Conclusion.

Mongolian spot cell and blue nevus cell belong to the same species and they are surmised to be the cells originated from mesoderma. Thus these are different from nevus cells of pigmented nevi which is neuroectodermal.

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Studies on Melanin.

VIII. Melanodermatitis faciei.

By

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(Received for publication, April 22, 1949)

Owing to the abnormal conditions of life under the World War II, it was quite naturally expected that unusual dermatoses are to be seen. Our melanosis is a specimen in point. What we call melanosis, first appearing during the war and still prevalent, is a peculiar pigmentary dermatose which frequents mosyly among females and is seen only in exceptional instances in the male. The affected area is chiefly face and neck. The skin color, unlike that of chloasma, varies from slaty blue to dark brown, and the number of the patients amounted to 68 and is still increasing. Such pigmentary disturbance was observed in Japan not only in our clinic but also in many other clinics. The cause has thus far been unknown and only disposition plus irritation through coarse cosmetics has been vaguely suspected. We have been studying its etiology since 1941 and have finally come to a conclusion.

We do not know whether melanosis were so frequently seen in Europe except the cases of Storck, Lutz and Garnier. In America Fiesler, Spiegel, Appel, Felsher, Rothman, Curth and Andrews and other authors have reported similar melanosis.

Historical Review.

During the past World War, Riehl of the Vienna school reported a sort of melanoderma which is known as Riehl's melanosis. In the following year Hoffmann and Habermann described melanodermitis toxica lichenoides et bullosa. The great resemblance of these two dermatoses with ours as well as reported cases in Japan is accepted by all Japanese authorities. Even the similarity of the two above mentioned dermatoses, however, has since been eagarly discussed. Hoffmann tried to hold Riehl's cases to his own, while Riehl insisting upon the autonomy of his own cases.

Now let us compare Riehl's cases with Hoffmann's according to the exhaustive descriptions of Kerl[1] and Habermann[2] on the German literature.

Kerl takes it as a very rare disease, the frequency of which is only fifteen to fifty thousand. Habermann, on the contrary, emphasizes the resemblance with already known dermatoses from tar or lubricating oil. As to its etiology, Kerl excludes all possible exogenous factors while Habermann admits external origin of his cases and emphasizes fluorescent acridine derivatives as causative agents.

Kerl concludes that Riehl's melanosis is caused by a toxemia of nutritional origin, probably resulting from the intake of dopa-like substances in vicia fava. But, the fact is, neither by feeding animals with horse beens alone nor by injection of unoxidated dopa solution can man see pigment formation. Habermann attributed hyperkeratotic and follicular type to external origin and apart from these ascribed pigmentary form to photosensitive substances accumulated by inhalation.

As to clinical symptoms, Kerl could easily distinguish his melanosis from others and emphasized its gradual formation, exclusive involvement of the face and the sculp, predominance of pigmentation, the absence of inflammatory changes and the histological intactness of the epidermis. But as he must later enlarge his category, these characteristics cannot be absolute. The naming of melanosis has since been much confused. Some authors reported melanosis of apparent occupational origin under the title of Riehl's melanosis. Sharlit is of opinion that melanosis with dermal pigmentation should be summarized under the title of Riehl's melanosis.

In our cases, some resemble Riehl's cases, others with Habermann's and between them are many undistinguishable ones. In fact some show much resemblance with pellagra or pellagroid dermatoses, some look like poikiloderma or erythematodes, while others show slaty bluish pigmentation only and some show mostly hyperkeratotic follicles and comedos—all these can be arranged in series. We then take it rational to summarize all pigmentary and hyperkeratotic and comedo-forming types under the common title of Melanodermatitis faciei. Especially in one comedo-forming type I could produce pigmentation with causative cold-cream, and in one pigmentary form comedo was the first complaint and pigmentation appeared later. These facts show that they have common etiology and have only conditional differences. But here for convenience, we divide them into three cardinal types according to three main symptoms—into pigmentary, comedo-forming and keratotic types.

Report of Cases.

Age, date of onset, proximate causes and prodroms are shown in the following tables. Among 68 cases only 3 were male patient.

TABLE I. Age.

	Under 20	20–29	30–39	40–49	50–59	over 60	Total
Pigmentary	0	11	10	6	4	1	32
Comedo-forming	1	20	5	4	1	0	31
Keratotic	0	3	1	1	0	0	5

TABLE II. Onset.

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Okt.	Nov.	Dec.
Pigmentary	1	3	3	2	2	2	2	8	2	1	1	1
Comedo	4	4	2	2	1	4	1	5	4	0	2	0
Keratotic	0	1	0	0	1	1	1	1	0	0	0	0

From these tables are shown that comedo-forming and keratotic types are seen most frequently in young females, while pigmentary form can occur in women over 30 years of age.

The frequent occurrence of the first type in August will be later mentioned.

Prodroms. Itching is the most frequent prodrom and is complained in each type. Erythema, and desquamation and swelling, however, are not noted in the comedo-forming and keratotic type. Noteworthy is the fact that in one case of the first type, comedo-formation was seen first and that the pigmentation appeared later on.

Proximate cause. Many substances are blamed as causative agents, of which the commonest one is cosmetics. In the pigmentary cases, sunlight and other actinic rays are also blamed.

Clinical symptoms. Brief summary of the clinical symptoms of the three types are as follows.

1. Pigmentary form. The frequent occurrence during the warmer time of the year was noted above. This helps me to assume photosensitization through coarse cosmetics. Freund saw pigmentation following the use of eau d'cologne only after irradiation by sun-light, and later perspiration was found to play a part in its etiology. The same relationship to actinic rays must be emphasized in my cases, too. Sometimes prodroms as erythema, swelling, itching and desquamation are complained. In one case such fits were the first complaint and pigmentation grew darker after each fit.

Hormonal disturbances and hypotonia, positive Aschner's reaction, as are often observed, indicate the instability of endocrine and autonomy nervous system. In most of them iodic acid value of the blood serum is markedly enhanced.

As regarding to the localisation, temporal, preauricular and mental region never fail to show pigmentation. Other parts of the face, retroauricular portion and the sides of the neck are also usually involved. In

three cases " butterfly-form " of the skin lesion was noted. The skin-colour varies from light slaty blue to dark violet, but unlike that of chloasma, tone of violet is always ascertainable. The skin surface in some cases is as coares as a grater, in others rather smooth and looks like powdered. Sometimes flat elevations of the skin fields are seen, but typical lichen planus-like papule is found by none. Erythema is noticed only in initial cases. By first glance, tanning forms rather diffuse patches, but on closer observation is found either in reticulated arrangement corresponding to the superficial network of blood vessels, or situated perifollicular. Direct surroundings of the follicular openings are rather free from tanning. Atrophy was not markable and was seen only in exceptional cases. In three cases the sculp was involved, in one of which tinea amiantacea-like enclosement of the hair was found.

Pigment macules lose their intensity at retroauricular region and diminish into tiny follicular patches. Finally they extinguish on the borderline to breast. Other parts of the body are usually free from tanning, except in one who carried pigment patches on the flexor surface of forearms. In some cases, pigmentation on the conjunctiva and on the buccal mucous membrane was noted, but yellow discloIration of the sclera was not seen. Amongst the 32 cases of pigmentary type are three intermediate ones which in addition to slight smutty pigmentation on the temporary region, showed follicular keratotic pluggings and comedos. In these three cases perifollicular infiltration was noted histologically. And pigment cells were seen chiefly in the follicular vicinity. Tendency to seborrhea was complained by all.

2. Comedo form. Forehead is rather slightly affected. Outer part of the eyebrows, zygomatic region, sides of the cheeks and mandibular angles are closely laden with a band of follicular papules, pustules and especially with black or skin-colored horny pluggings. Pigmentation, if any, is not prominent and is locallized mostly near follicular openings. Pigment cells are not found microscopically.

3. Keratotic form. Both sides of the cheeks, preauricular region are chiefly affected. Even the nape of the neck was affected in one. Each papule is almost as large as rice grain, follicular located and is usually skin-colored but sometimes livid violet, abnormally hard and renders the skin the feeling of a grater. In typical cases almost similar exanthemas are seen, but in some pustules are mixed.

This type visited us successively in summer of 1945, and were successfully treated with cod's liver oil or butter intake. This suggests us that this type had occured upon the deficiency of vitamin A and D. The dark adaptation of these cases, however, was not examined because they failed to visit us till several weeks later, when the skin was entirely clear of erup-

tion. Although vitamin A and D are reported to be effective in acne vulgaris, we could not get successful results in comedo forms as in this.

Histopathology and histochemical findings. In eight cases biopsy specimens were excised. They are obtained from six pigmentary cases containing one intermediate case and two comedo-forming cases.

Specimen No. 1. Section obtained from a female patient of 31 years of age complaining pigmentation of the face for two months. Epidermis is thinner than usual with shorter processus. Basal layer is laden with little pigment. In the papillary and subpapillary region, espec. near the hair follicles, are situated pigment cells close to blood vessels. Melanin granules are seen also in reticulum cells. Inflammatory changes have utter subsided.

Specimen No. 2. Obtained from a 30 years old woman. Duration of the pigmentation more than five years. Thin epidermis. No hyperkeratosis. Some intraepidermal vacuoles are found. Perivascular infiltrate in the upper dermal layer consists of lymphocytes and hictiocytic cells. Chromatophores are increased in number. Melanin granules are phagocyted in the reticulum cells, too. Elastic fiber is lessened in the infiltrated parts.

Specimen No. 3. Section obtained from a 37 years old female patient with the pigmentation of the face of three years duration. Epidermis is slightly parakeratotic. Rete thickened. Basal layer is poor in pigment. In the corium perivascular edema and infiltrats of round and histiocytic cells are noticed. These reach to the middle layer of the corium. Pigment cells are distributed unevently in the papillary and subpapillary layer.

Specimen No. 4. This section was excised from a 29 years old female patient with severe dark brown pigmentation of the face of two months duration. Epidermis is somewhat disordered and vacuolated near follicular openings. The basal layer shows subnormal pigment content. Hair follicles strongly dilated and filled with horny masses. In the corium are seen moderate perivascular and perifollicular infiltrates cosisting of round and histiocytic cells in the subpapillary layer, where numerous melanophores are found especially at the vascular surroundings.

Specimen No. 5. Section obtained from a 28 years old female patient. Duration of pigmentary disturbance 4 months. Rete cells vacuolated and loosely connected. Borderline to the corium not distinct. Dilation of follicular openings are remarkable. Here and there intraepidermal cellular infiltrates are noticed. Basal layer contains subnormal amount of melanin granules. Numerous pigment cells are situated in the papillary layer. In the deeper layer of the derma, round and histiocytic cells and chromatophores are seen in the vicinty of blood vessels.

Section No. 6. was excised from a 54 years old female with pigmentation of one years duration. Pigment content of the basal layer is some-

what increased. Follicular openings are strongly dilated, in which fat and horny masses are found. In the corium are seen hyperemia in the upper layer and round cell infiltration in the surroundings of blood vessels, where pigment is situated in melanophores and in connective tissue fibers. Marked edema and cellular infiltration are seen in the follicular vicinity.

Specimen No. 7. Excised from a 30 years old woman complaining itching and erythema and comedo formation on both cheeks for about three months. Epidermis is thinner than usual and no epidermal processus are seen. Basal layer contains normal amount of pigment. No pigment cells in the derma. In the subpapillary layer blood vessel are dilated and edematous. Hair follicles are expanded, contain comedos. The basal layer of hair follicles is rich in pigment. Degeneration of arrector muscle is noted.

Specimen No. 8 was obtained from a 22 years old female patient, who after the use of a cold-cream for a short time, noticed numerous comedos and pustules on both cheeks. Miscroscopically epidermis is flattened at follicular openings. Basal layer, which is rich in melanin, is somewhat disordered. Apparent hyperemia in the upper dermal layer is noted. Cystic enlargement of hair follicles is seen even macroscopically. The tissues surrounding them are pressed aside, but show little or no inflammatory changes.

Brief summary of the histo-pathological findings is as followes.

In the first type,the epidermis shows various stages of degenerative changes according to the clinical signes of acuity. Common characteristics lies in the diminished or no pigment of the basal layer and in the presence of various numbers of pigment cells in the upper dermal layer. In most of the cases perivascular infiltrates consisting of round and histiocytic cells are seen. The affection of the follicle apparatus differs by cases, but, generally speaking, grows severer as the clinical appearance of comedo takes place.

In the comedo-forming type the main aspect lies in the utmost dilatation of hair follicles.

In all these specimens are noted four cardinal pathological features--degeneration of epidermis, affection of hair follicles, increase of pigment cells in the corium and inflammatory changes in the corium. And it is made clear that they show gradual passing from one type to another.

In the specimen No. 3, I stained the section with dopa and got, unexpectedly, positive results. The author tried the dopa-reaction after the variation of Fujiwara,[3] and found that after 90 minutes in 37°C. small pigment cells of the upper dermal layer undoubtedly darkened. As the free melanin granules in that layer are not stained black, it is obvious that there is no absorption of oxydated dioxyphenylalanin by melanin granules.

The dopa-reaction of Bloch[4] has been estimated to be an indicator of the pigment forming facility of a cell, which has since been attributed only to the dendritic cells of the basal layer and to dermal melanoblasts in certain cases. But the true nature of this reaction is still under discussion. Especially the discussions of Meirowsky and Katsunuma are not throughly answered. So, in order to confirm our results, we further stained two sections with peroxydese-reagence and with Unna's oxygen-space-method. The results were quite corresponding—that is, by both Unna's and Okano's[5] methode dermal pigment cells were found to contain peroxydase granules, and that the less the melanin content, the more the peroxydase just as Watanabe[6] in our clinic proved in several pigment producing tissues. And by Unna's oxygen-space-method, infiltrated parts of dermal layer is stained deep green as oxygen space and especially strongly stained is the vicinity of blood vessels—in short, where the pigment cells are located, there is ample amount of free oxygen.

Epiology and Pathogenesis.

With regards to the etiology of this dermatose, we will take up photosensitisation through coarse cosmetics as external factor and hypovitaminosis and hormonal disturbances as internal, and as a supplementary factor dysharmony of the autonomy nervous system.

Hoffmann and Habermann ascribed hyperkeratotic form and folliculitis in his cases to external contact with petroleum and pitch, and apart from these, contributed pigmentary form—melanodermitis in the sence of the word—to inhalation of toxic substances. Riehl and Kerl attached great importance to the nutritional origin of their cases and excluded all possible external irritants. Most of our cases are women of indoor activities having no occupational contact with tar, pitch or lubricating oil. Professional causes can be distinctly excluded. Neither can nutrition alone explain such occurrance. So it is quite natural that most Japanese authors took up crude cosmetics as causative agents. Shortly after the War, Tanimura and Hayami reported acne-like eruption from the use of cold-cream. But as to the irritants in it, the former finds cause in insaturated fatty acids, while the latter in lower hydrocarbons. Recently Kitamura[7] blamed fluorescent substance contained in oil used for cooking as a cause of melanosis. We analysed cosmetics obtained from patients. All have flavour of bergamotte oil, and in three of them lower hydrocarbons were found to be the chief component. These bergamotte oil and lower hydrocarbons we take up as external causative agents.

It is well admitted that spread of fat or oil on the skin increases pigmentation by irradiation. Nakajo[8] in our clinic showed the inhibitory action of vitamin C and B_2 against the pigmentation by ultraviolet irradia-

tion after tar or bergamotte oil spread. According to his theory, this action is due to the decreased permeability of the skin by vitamin C and B_2. So it is quite naturally expected that in B_2 or C- hypovitaminotic individuals the skin is much more susceptible to external irritants (in this case, to bergamotte oil or lower hydrocarbons) than normal persons. In fact, the vitamin C content of the patients' skin measured by 2, 6, dichlorphenolindophenol-method is distinctly lower than normal controls. From this is clear that the hypovitaminitic stage induced by the deficient supply of nourishment during the war, enhanced the susceptibility of the skin against various photosensitizing substances.

On the other hand, vitamin C exerts inhibitory action on the pigment formation as shown by the supressive effects to dopa-reaction and to the peroxydase reaction. That is, vitamin C deficiency contributes to the etiology of this disease in duplicated way—first by enhancing the penetrability of the skin, secondly by the loss of inhibitory action against pigment production. Cutaneous manifestation of vitamin C deficiency is known as scurvey, but its former stage, together with anorexia, arthralgia and tachycardia, shows follicular hyperkeratosis and pigmentation. But as general complaints were rare in our cases, ours is not true hypovitaminosis or dysvitaminosis, but a sort of paravitaminosis in Comel's[9] sence.

As to vitamin A and D, Kitamura obtained no definitive results in dark adaptation in his cases. Hypovitaminosisacne of American authors[10] may have resemblance, but general dryness of the skin was not observed in our cases except in one who carried slight follicular keratosis on the extensor surface of extremities. However, the successful treatment with cod's liver oil shows the participation of these vitamins at least in pure keratotic type.

In the former parts we admitted possible photosensitization through bergamotte oil or lower hydrocarbons or acridine derivatives. But, here too, the participation of another vitamin cannot be overlooked. Ito[11] could cure pellagra with intake of vitamin B-complex and later with vitamin B_1 alone. Later vitamin B-complex deficiency was found to cause pellagra or pellagroid dermatose. In some of our cases, the lesions were localized on both cheeks in butterfly-form accompanied with other clinical signs of vitamin B-complex deficiency. Hasebroek also reported similar case in his melanosis. Such facts suggest to us the participation of vitamin B-complex through successful treatment with this alone was inprocurable. In short vitamin B deficiency must have taken part in this disease accompanied with the enhancement of the permeability of the skin.

As to the relationship of autonomy nervous system with the pigment formation, Ito has already made exhaustive researches which make it unnecessary to touch here again.

The role of adrenals and ovaries in case of melanosis was noted by Comel and Rolba. The irregularity of menstrual cycles was usually complained in pigmentary cases. Recently Suzue and Yamamoto[12] reported a case of vagabond's disease with extensive degeneration of adrenals. Some of our cases showed hypotonia and pigmentation on the buccal mucous menbrane and were successfully treated with prolonged injection of the cortical horman of adrenals. From these facts the role of the endocrine system cannot be overlooked.

Pathogenesis. The point of discussion between Bloch's school and Meirowsky[13] lies in the pigment forming facilities of superficial pigment cells in the corium in man. These pigment cells increase much in number in cases of melanosis and form the greatest characteristics. In all reported histopathology of melanosis, these cells are dopanegative and are attributed to no melanogenic activities, except that of Shwartz's case who admitted " primary activity in the CHROMATOPHORES."

Meirowsky, though he admits the negative dopa-reaction of the corium cells, persists on the mesodermal origin of melanin, supported by Lipschütz's animal experiments. Judging from our histologic and histochemical findings, the pigment content of the derma never goes parallel with that of the epidermis. The abundant store of melanin by almost intact epidermis cannot be explained by Bloch's theory only. Besides, the assumption of the loss of pigment retaining facility of epidermal cells is too hypothetic.

From above mentioned researches the author concludes as follows: Owing to the enhanced permeability of the skin induced by vitamin C and B-complex deficiency, the component of crude cosmetics, lower hydrocarbons reaches corium. Where with the aid of vitamin B-complex deficiency it causes photosensitization and, as the results, abnormal oxygen-space is produsedin derma. But in some cases, where perivascular region is especially rich in oxygen, the hematogenous distribution of photocatalytic substances cannot be excluded.

Oizumu[14] found higher content of iodic acid reducing substances in blood serum in cases of pigmentary form. This suggests the possibility that both premelanin—tryptophane or tyrosine—and catalyser reach derma through blood vessels. And the positive peroxydase reaction. Though peroxydase does not always coincides with oxygen-space, oxygen-space usually contains peroxydase. Thus the oxidizing potency is extremely enhanced and then the pigment formation takes place in the presence of peroxydase. Whether the peroxydase concerned is of true fermentative nature or only pseudoperoxydase, it does not matter. And where the oxidizing potency is high and pigment production takes place, positive dopa-reaction is naturally expected. Thus the more rational dermal pigment formation in our melanosis is explained.

In cases where follicular eruptions are predominant, the invade of cosmetics takes place chiefly through pilous poles, and cause acnelike eruption or, supported by the deficiency of vitamin A, cause hyperkeratotic papules. Even in such cases pigment formation may occur as shown in the intermediate cases.

Summary and Conclusions.

After the historical review of melanosis, 68 cases of melanodermatitis observed in Tohoku University were reported.

From the clinical and histological observations they were divided into three types. The characteristics of each type is discribed. All these types have a common etiology and have only conditional differences.

As to the etiology, the role of subclinical vitamin deficiencies and irritative cosmetics was emphasized. In one case dermal pigment formation was certified with positive dopa- and peroxydase-reaction.

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Results of Cultivation of Tubercle Bacilli from Blood Stream, especially after Operation, in Surgical Tuberculosis.

By

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We cultivated tubercle bacilli from the blood stream by the methods of Löwenstein[1] and Iibuchi,[2] applying the culture media of Löwenstein and Oka-Katakura,[3] usually twice, but often 3 to 5 times in each of the 270 cases of several surgical tuberculosis before operation and obtained the results shown in Table I. Cultivation of tubercle bacilli was positive in 3 cases. The clinical conditions were in these 3 cases very severe, showing complications of serious pathological findings in x-ray photograph of the lung in 2 cases and of severe genito-urinary tuberculosis in one case.

TABLE I.

Results of Tubercle Bacilli Cultivation.

Diseases	Tests without and before operation			Tests after operation		
	Test cases	Positive cases		Test cases	Positive cases	
		Number	Percent		Number	Percent
Renal tbc.	94	2	2	69	4	6
Chestwall tbc.	43	0	0	43	0	0
Pulmonal tbc.	10	1	10	6	1	17
Lymphgland tbc.	41	0	0	41	0	0
Epididymis tbc.	37	0	0	37	1	3
Anal fistula	30	0	0	30	0	0
Peritoneal tbc.	8	0	0	6	0	0
Ossal tbc.	7	0	0	4	0	0
Total	270	3	1	236	6	3

236 of the 270 cases, which showed negative bacilli cultivation of the blood, were operated. Tubercle bacilli were cultured from the blood in these cases usually 5 times, namely on the first, third and seventh day,

second and third week after operation and in longer treated patients, further once in 2 weeks thereafter. In the cases with complication of high fever after operation the cultivation tests were made continuously every third or fourth day.

After operation bacteriemia was found in 6 cases, namely in 4 cases after nephrectomy for renal tuberculosis, in one case of gastric ulcer with complication of pulmonal tuberculosis after resection of the stomach and in one case of genito-urinary tuberculosis after epididymectomy. These 6 cases with bacteriemia died within 20 to 107 days after operation due to tuberculous meningitis or miliary tuberculosis. Fatal aggravation of tuberculosis after operation was found in 11 cases, among which the cultivation was always negative in 5 cases in spite of repeated examinations. Cultivation of bacilli was always negative in the 189 cases of favorable postoperative conditions and in the 11 cases with complication of transitory aggravation of pulmonal tuberculosis after operation.

The percentage of positive tubercle bacilli cultivation in surgical tuberculosis has been reported by many authors, for instance, Löwenstein[4] records 59% in the case of renal tuberculosis, Iibuchi[3] claims 25%, and some authors[5-9] state only 5 to 8% or even 0%. Bacteriemia was found in 1% of the 270 cases before and in 3% of the 236 cases after operation, therefore the percentage of positive bacteriemia in our cases was smaller than that of certain authors, while the number of cases examined exceeded that of previous authors. Postoperative bacteriemia was found only in a few cases accompanied with progressive severe complications of tuberculosis. The results of examination by employing the methods of both Löwenstein and Iibuchi were the same, and the same can be said for the applied culture media of both Löwenstein and Oka-Katakura.

Edwin[10] described that tuberculous infection of nephrectomy wound for renal tuberculosis is caused by tuberculous bacteriemia after operation. In the 29 cases of tuberculous infection of the wound among 69 nephrectomies, 2 cases of tuberculous bacteriemia were found with fatal aggravation of tuberculosis after operation. Therefore we disagree that tuberculous infection of nephrectomy wound is generally caused by tuberculous bacteriemia after operation. A discussion will be presented after a better and more reliable method of this cultivation from blood has been found.

Conclusion.

Tubercle bacilli were cultivated from the blood stream by the methods of Löwenstein and Iibuchi in 270 cases of various surgical tuberculosis before operation. More than 2400 cultivations were made on 236 cases after operation. Tuberculous bacteriemia was observed in 3 cases (1% of 270 cases) before and in 6 cases (3% of 236 cases) after operation. These cases

with bacteriemia were accompanied with serious progressive complications of tuberculosis.

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A Method to Take Radiograms of the Transsection of the Body at any Inclination and Curvature*

Preliminary Report.

By

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We prepared two rotationtables A and B. On the rotationtable A a patient was seated and on the rotationtable B a film placed. We arranged a tubefocus and rotationaxes of two rotation tables in a vertical plane. The tubefocus was situated a little upwards so that the central x-ray inclined to the table of the rotationtable B about 15°. In order to remove the useless but injurious x-ray a pair of lead slits and a Bucky-Potters diaphragm were equipped at the front of the patient. A horizontal film was laid on the rotationtable B. The x-ray switch was put on and the two rotation tables rotated synchron by the electric motor from 0° to 180°. The film obtained is imaged with the horizontal transsection of the body. When we place a film inclined or when we place a film packed in a curved cassette on the rotationtable, then we get a film imaged with an inclined or curved transsection of the body. Here the body is transsected by the plane which is parallel to the film and contains the point, dividing the line from the tubefocus to the film in the ratio of tuberfocus-rotationtable A—distance to the rotationtable A and B distance. The thickness of the layer, imaged on the film, is controlled by the inclination of the central x-ray to the rotationtable B at this radiography.

Inclination:	3°25′	8°45′	32°
Thickness of the layer:	0.41mm	0.82mm	1.23–1.64mm

These results were proved not only geometrically, but also experimentally.

Now we are taking rotating crossgrams of the human body at any inclination and any curvature. We show three leaves of crossgrams of the chest. First. A horizontal crossgram of a female aged 25. at the height of the hilum. (This was exposed at 80 KV, 28 mA, 5 seconds.; tuberfocus-body distance; 187 cm; body-film distance, 48 cm.) Second. A crossgram of a male aged 27. The film was inclined 40° to the level. The transsection, directed from upper back-hilum-lower front, was imaged. Third. A crossgram of a male aged 27. The film was curved in parallel to the curvature of the right diaphragm.

* We reported this at the first meeting of the Hirosaki Roentgen Society, Dec. 25th, 1949 (Hirosaki Ig. Z. Vol. 2, No. 1). Thereafter in Feb. 1950 we received from Dr. A. W. Pryde a reprint of a paper of Dr. Gebauer who had worked along a similar line in 1949.

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Blood Picture of Apparently and Really Healthy Children.

62nd Hematological Paper.

By

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Reports on the blood picture of healthy children are not rare in this country as well as in foreign ones. But the "healthy" children in this treatise—older children are meant here—are rather prize boys and girls selected from among many children from different parts of this prefecture. They were not only superior in dimensions of the body but also in bodily and mental performances. These first rate children were now submitted to blood and X-ray examination. The present treatise is the result of this investigation.

There were 47 boys and 44 girls, 91 children in all. They were 12 years of age at the time of examination.

Blood was taken before noon, and blood films were stained by the Tohoku-Pediatrics Method[1] (a modification of Sato and Sekiya's Peroxidase Reaction[2]). Hemosedimentation was determined by Yoshida's micro-method.[3] The blood counts obtained were judged according to the Standard Table of Sato and Suzuki.[4] All the children were examined rentgenologically in the Department of Radiology. Through this examination 7 children were excluded from among the 91 children on account of suspected active tuberculosis.

Results.

1. Red count (Table I). Red counts and hemoglobin were within the normal range: No case with an anemia.

2. White count (Table II). The result was rather beyond expectation, because only 1/3 of the cases showed a normal count of 6,000–8,000. 11 children (13.1% of all the cases) showed a leucocytotic count over 9,000 cells. A rather striking leucocytosis was seen in 8 children out of these 11.

TABLE I.

Frequency Distribution of 84 Cases according to Red Count (Thousands).

Sex		~4000	~4500	~5500	~6000	6000~
♂	Cases (%)	3 (7.1)	8 (19.0)	25 (59.6)	4 (9.5)	2 (4.8)
♀	„ („)	7 (16.7)	7 (16.7)	23 (54.7)	1 (2.4)	4 (9.5)
Total	„ („)	10 (11.9)	15 (17.8)	48 (57.2)	5 (6.0)	6 (7.1)

TABLE II.

Frequency Distribution of 84 Cases according to White Count.

Sex		~4000	~5000	~6000	~8000	~9000	~10000	10000~
♂	Case (%)	2 (4.5)	9 (21.6)	5 (11.9)	13 (30.8)	5 (11.9)	3 (7.1)	5 (11.9)
♀	„ („)	3 (7.1)	7 (16.7)	10 (23.8)	16 (38.1)	3 (7.1)	1 (2.4)	2 (4.8)
Total	„ („)	5 (6.0)	16 (19.0)	15 (17.9)	29 (34.5)	8 (9.5)	4 (4.8)	7 (8.3)

TABLE III.

Frequency Distribution of 84 Cases according to Neutrophil Count.

Sex		~1500	~2000	~3000	~3500	~4200	~5000	~6000	~7000	7000~
♂	Cases (%)	2 (4.8)	10 (23.9)	7 (16.7)	4 (9.5)	4 (9.5)	3 (7.1)	4 (9.5)	4 (9.5)	4 (9.0)
♀	„ („)	0 (0)	13 (31.0)	6 (14.2)	13 (31.0)	13 (31.0)	1 (2.4)	1 (2.4)	1 (2.4)	1 (2.4)
Total	„ („)	2 (2.3)	23 (27.2)	13 (15.5)	17 (20.2)	17 (20.2)	4 (4.8)	5 (6.0)	5 (6.0)	5 (6.0)

TABLE IV.

Frequency Distribution of 84 Cases according to Lymphocyte Count.

Sex		~1000	~1500	~2000	~2500	~3000	~4000	4000~
♂	Cases (%)	1 (2.4)	7 (16.7)	6 (14.3)	15 (35.6)	6 (14.3)	5 (12.0)	2 (4.8)
♀	„ („)	1 (2.4)	1 (2.4)	11 (26.2)	11 (26.2)	11 (26.2)	5 (12.0)	2 (4.8)
Total	„ („)	2 (2.4)	8 (9.5)	17 (20.2)	26 (31.0)	17 (20.2)	10 (11.9)	4 (4.8)

TABLE V.

Frequency Distribution of 84 Cases according to Micro Hemosedimentation (mm).

Sex		2–5	6–11	11–13	13–
♂	Cases (%)	25 (59.5)	13 (31.0)	4 (9.5)	0 (0)
♀	„ („)	31 (73.8)	10 (23.8)	1 (2.4)	0 (0)
Total	„ („)	56 (66.6)	23 (27.4)	5 (6.0)	0 (0)

TABLE VI.

Frequency Distribution of 84 Cases according to Percentage of "Stabkernig" Neutrophils.

Sex		10%	20%	30%	30%
♂	Cases (%)	26 (61.0)	14 (33.4)	0 (0)	2 (4.8)
♀	„ („)	29 (68.8)	9 (21.6)	2 (4.8)	2 (4.8)
Total	„ („)	55 (65.6)	23 (27.2)	2 (2.4)	4 (4.8)

TABLE VII.

Frequency Distribution of 91 Cases according to X-ray Examination.

Sex		"Cured"	Old pleuritis	Suspected pleurisy	Active *tbc* of lungs	Negative
♂	Cases (%)	10 (21.3)	0 (0)	0 (0)	5 (10.6)	32 (68.1)
♀	„ („)	7 (15.2)	1 (2.3)	1 (2.3)	2 (4.5)	33 (75.7)
Total	„ („)	17 (18.7)	1 (1.1)	1 (1.1)	7 (7.7)	65 (71.4)

TABLE VIII.

Mean Value of Blood Counts of the 56 Children with Negative X-ray Finding.

Sex	Red count (thousands)	Hemoglobin (Sahli value)	White count	Basophils	Eosinophils	Neutrophils	Monocytes	Lymphocytes	Micro hemosedimentation	"Stabkernig" (%)
Boys	4980	87	8650	30	280	3610	400	2250	5.5	8.5
Girls	4890	85	6610	30	260	3500	350	2530	4.5	10.0
Mean	4930	86	6630	30	270	3550	370	2390	5.0	9.3
Round	5000 (±500)	70–80 (±5)	7000 (±1000)	0–60	150–250	3500 ~4200	300 ~500	2000 ~2500	♂ 2–4 ♀ 3–5	

3. Neutrophils (Table III). 17 cases (18.7%)—13 girls among them—showed a normal count. A neutrophilia (over 6,000 cells) was observed in 8 boys and 2 girls. A neutropenia (under 2,000 cells) occurred in 6 boys and 4 girls.

4. Lymphocytes (Table IV). 26 children—thus 1/3 of all the cases—showed a normal count and there was almost no difference according to the sex. A lymphopenia (under 1,000 cells) was seen in a boy and in a girl. A lymphocytosis (over 2,500 cells) occurred in 13 boys (31.1% of all the boys) and in 18 girls (43.8% of all the girls).

5. Eosinophils and monocytes: They showed a normal value.

6. Hemosedimentation (Table V). Normal in 56 cases. A moderate acceleration was seen in 4 boys and 1 girl.

7. Nuclear shift (Table VI). Assuming that the upper limit of the normal range be 10% or so, 2/3 (or 65%) of all the cases were of a normal percentage. In 14 boys and 9 girls, thus in 23 cases (27.2%) a slight shift to the left was seen. In 2 girls a striking left shift occurred. A pathologically severe left shift was observed in 2 boys and 2 girls.

8. Radiologic examination (Table VII). Suspected active tuberculosis of the lung was observed in 7 cases (5 boys and 2 girls) and an old lesion (at least presumptively so considered) in 19 cases (20.8%).

Comment.

All in all, red count did not show an abnormal value, but as to white count a leucocytosis occurred in 11 cases (13.1%) and a neutrophilia in 10 cases (12%). And these 10 cases belonged to those 11 cases with leucocytosis. A large majority of these were boys (cf. Table VIII).

Hemosedimentation was moderately accelerated in 5 cases (6%) (4 boys and 1 girl). As to the nuclear shift a remarkable shift to the left was observed in 6 cases (7.2%). And in selected "healthy" children occurred a leucocytosis due to neutrophilia. Besides, active tuberculosis was identified in 7 cases. If these cases and those of leucocytosis over 9,000 cells and those of leucopenia below 5,000 cells are excepted, then the mean value of hematological figures of the 55 healthy children are in good accord with those in Sato and Suzuki's Standard Table[4] for Every Age.

Conclusions.

There were not a few unhealthy children among even selected "healthy" ones, if submitted to hematological and radiological examinations.

Literature.

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2) A. Sato and Sekiya, Ibid., 1926, **7**, 111.
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A Method to Take a Radiogram of the Body in Three Dimensions.

Preliminary Report.

By

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(Received for publication, March 27, 1950)

We prepared two rotationtables A and B. On the rotationtable A a patient is seated and on the rotationtable B horizontal sheets of films placed, being piled to the cubic block. We arrange a tubefocus and rotationaxes of two rotationtables in a vertical plane. The tubefocus is placed a little upwards so that the central x-ray inclines to the table of rotationtable B about 15°. When we put on a x-ray switch and rotate two rotationtables synchron by the electric motor from 0° to 360°, then we get the x-ray image of the body in the film block in three dimensions. The x-ray image in the film block is similar to the actual body but enlarged in the ratio of tubefocus-rotationtable B distance to the tubefocus-rotationtable A distance. These were proved not only geometrically but also experimentally. In the experiment a model was employed by piling two eggshaped barium blocks (the left white body in the figure). Here a mirrow is used in this figure to get a back view. We solidographed this model. Then we unbound several sheets of films and developed. After cutting out the figures appearing on the film, we piled these again and plastered. We got an x-ray image of film block in black (the right body in the figure). Thus we could demonstrate actually that by this operation an x-ray image of the body in three dimensions is obtainable on the rotationtable B. We are now taking solidograms of the chest in the living human body.

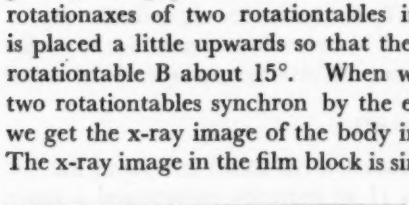

Fig. 1. A model (white) and a solidogram (black). By a mirror back sides are reflected.

The Tohoku Journal of Experimental Medicine, Vol. 52, Nos. 1 & 2, 1950.

Biochemical Studies on Carbohydrates.

CXXVII. Structural Study of A-specific Carbohydrate from Mucus Mucin of Pig Stomach.

Third Paper.

By

Zensaku Yosizawa.

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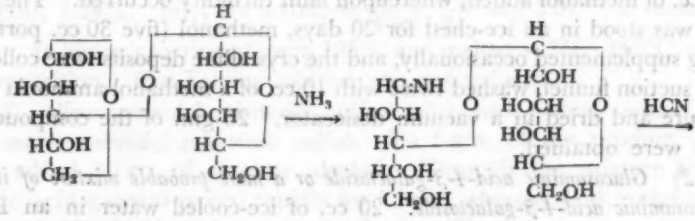
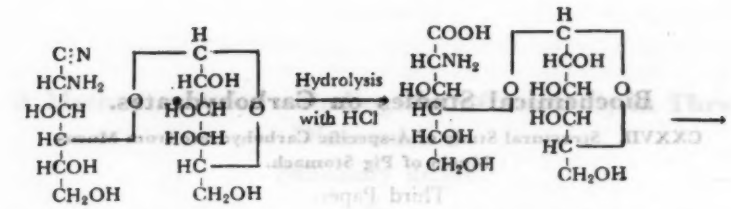
(From the Medico-Chemical Institute, Tohoku University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, May 23, 1949)

The experiments in the second report[1] indicated that the unit disaccharide of the carbohydrate above is N-acetylglucosamine(1,5)-4-β-galactoside(1,5), if its monosaccharide components both take the pyranose form. This structure has been firmly established by synthesis which was started with lactose. The material was changed into *d*-arabinose-3-β-galactoside (I) according to Zemplén[2] and followingly, by the procedure of Fischer and Leuchs[3] for preparing glucosamine from *d*-arabinose, into glucosamine-4-β-galactoside (VI) through *d*-arabinosimine-3-β-galactoside (II), glucosaminonitrile-4-β-galactoside (III) and glucosaminic acid-4-β-galactoside (IV) and its lactone (V) in turn. Complete N-acetylation was achieved by adapting the ketene method of Bergmann and Stern[4] at first and then the acetic anhydride-silver acetate method of Zuckerkandl and Messiner-Klebermass.[5] The product thus obtained was non-homogeneous, probably being a mixture of the epimers.[6] Hence it was fractioned patiently with organic solvents until a substance was separated that agreed not only in composition but also in physical properties with the natural disaccharide.

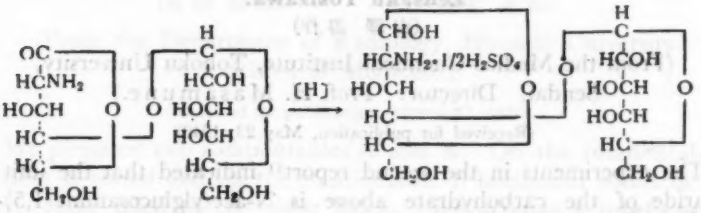


d-Arabinose-3-β-galactoside (I) *d*-Arabinosimine-3-β-galactoside (II)



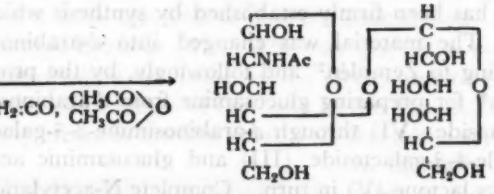
Glucosaminonitrile-4-β-galactoside (III)

Glucosaminic acid-4-β-galactoside (IV)



δ-Lactone (V)

Glucosamine-4-β-galactoside sulfate (VI)



Acetylglucosamine-4-β-galactoside (VII)

Experimental.

1) Arabinosimine-3-β-galactoside. 28 gm. of *d*-arabinose-3-β-galactoside[3] were suspended in 50 cc. of methanol and shaken with 250 cc. of 28% ammonia which were added from time to time in portions. Some insoluble part was filtered off, the filtrate diluted with 200 cc. of ether and 100 cc. of methanol added, whereupon faint turbidity occurred. The mixture was stood in an ice-chest for 20 days, methanol (five 30 cc. portions) being supplemented occasionally, and the crystalline deposits were collected on a suction funnel, washed twice with 10 cc. of a methanol-ammonia (1:1) mixture and dried in a vacuum desiccator. 26 gm. of the compound in view were obtained.

2) Glucosaminic acid-4-β-galactoside or a more probable mixture of it with mannosaminic acid-4-β-galactoside. 20 cc. of ice-cooled water in an Ellenmeyer flask were saturated with HCN-gas, the whole product above added and the mixture shaken as stoppered (ground joint). The sub-

stance dissolved gradually. The yellowish solution obtained was followingly heated for 30 minutes at 40°C, whereby it changed extremely viscid and, on passing HCN-gas for 30 minutes under cooling, it became again a thin liquid. After again heating in a like manner as above, the liquid thus made viscous was chilled (in a freezing mixture of ice and NaCl) and 100 cc. of concentrated HCl (sp. gr., 1.19), similarly cooled, mixed, whereupon turbidity appeared. The mixture was stood in air (3°–12°C) for 24 hours (it got colored greenish) and evaporated under reduced pressure to a dark green syrup. The saturated $Ba(OH)_2$ was added in excess (5 cc.), then the brown muddy solution submitted as such to distillation in vacuo to expel off ammonia and to the residue (syrup) were added 20 cc. of 1 n. H_2SO_4. On heating to 40°C, $BaSO_4$ subsided, which was filtered off, and the filtrate mixed with 20 gm. of lead oxide, heated on a boiling water-bath for 2 hours to throw down sulfuric and hydrochloric acid as lead salts, and filtered. The bulk on the filter was washed with two 20 cc. portions of water. Through the filtrate and washings combined was passed H_2S gas and precipitating lead sulfide separated off on a filter and washed twice with 10 cc. of water saturated with H_2S. The filtrate and washings here were evaporated together by vacuum distillation at first to about 20 cc. and after addition of 30 cc. of absolute alcohol, furtheron to a dough (green colored). The remainder was diluted with 6 cc. of water and spread on a porous plate. Crude glucosaminic acid-4-β-galactoside (brown, amorphous) on the plate was collected and dried up in a vacuum desiccator ($CaCl_2$). Crop, 16 gm. For the purpose of purification, the mass was dissolved in 50 cc. of water, treated with 1 gm. of charcoal on a boiling water-bath and filtered and the filtrate distilled in vacuo. There remained a yellowish porridge of fermlike crystals. On recrystallization from water, 12 gm. of almost non-colored hygroscopic crystals, which had the same appearance as on first crystallization, were yielded. For analysis, the product was dried in a vacuum desiccator over P_2O_5 to a constant weight. N (micro Kjeldahl), 3.76% (calc., 3.75%). Galactose 48.17% (indole method: 48.17%, orcinol method: 48.17%; calc., 48.26%). Ash (micro analysis), 1.24%. $[\alpha]_D^{11}$ (in water) $=\frac{0.573\times 100}{1\times 11.31}=+5.07°$ (no mutarotation). It began charring at some 125°C and melted at 156°–160°C under decomposition. Hot Fehling solution was not reduced.

3) Glucosamine-4-β-galactoside sulfate or a more probable mixture of it with mannosamine-4-β-galactoside sulfate. To 4 gm. of the product given were added 40 cc. of absolute alcohol. Through the mixture hydrochloric acid gas was passed rapidly for 1 hour, whereby heat evolved and nearly half of the substance dissolved. The suspension was then boiled on a water-bath under reflux for about 20 minutes.[7] When

cold, it was distilled almost to dryness in vacuo and the residue diluted with 20 cc. of absolute alcohol was again distilled to eliminate hydrochloric acid. The syrupy residue here—the lactone—was taken up in 30 cc. of water (A clear brown solution with greenish shade was obtained), frozen in a salt-ice mixture, acidified with 0.5 cc. of 10% sulfuric acid and shaken with 2.5 gm. of 2.5% sodium amalgam. When gas evolution ceased, the solution was again frozen, acidified and treated with the sodium amalgam. The operations were repeated until the amount of the sodium amalgam reached 25 gm. The precipitating mercury and sodium sulfate were separated by filtration and washed twice with 5 cc. of water. The filtrate and washings united (pH, 2.5) were adjusted to pH 4.0 by the aid of 10% KOH, heated with 1 gm. of charcoal on a water bath for 1 hour and the non-colored filtrate was condensed by vacuum distillation (Abundant crystals came out). The rest was submitted to similar evaporations with a 15 cc. portion of methanol and two 15 cc. portions of absolute alcohol to remove water completely. The remainder finally dried up was treated with 40 cc. of boiling absolute methanol, the extract filtered while hot and the unextracted part was washed with two 5 cc. portions of absolute methanol. The filtrate and washings were evaporated together, then the residue extracted with methanol (The extract was filtered when cold) and the unextracted part washed similar to above. On distilling of the extract and washings here, crystals in the form of twig were given. This crude glucosamine-4-β-galactoside sulfate[3] was recrystallized from methanol and dried constant in a vacuum desiccator (P_2O_5)—1.8 gm. The product was hygroscopic and non-colored and had the form as above.

N (micro Kjeldahl) 3.52% (calc., 3.59%), galactose 46.29% (indole method, 46.42%; orcinol method, 46.15%) (calc., 46.16%), ash (micro analysis) 0.07%. $[\alpha]_D^{11}$ (in water) $= \frac{0.317 \times 100}{1 \times 2.80} = +11.32°$ (no mutarotation). It began charring at about 107°C and melted at 125°–128°C under decomposition. Hot Fehling solution was reduced. In this test NH_3 was splitted off.

4) Acetylglucosamine-4-β-galactoside. 1.2 gm. of the above substance were taken up in 20 cc. of water, and after the pH of the solution was made 7.3–7.5 with the saturated $Ba(OH)_2$, ketene passed for 2 hours, the reaction of the solution being maintained at the same pH by frequent addition of baryta. Thereafter 10% H_2SO_4 was added to pH 4.0, precipitating $BaSO_4$ centrifuged off, and the supernatant liquid and washing (10 cc.) of the centrifugate were combined and distilled in vacuo to about 10 cc. The condensate was then acidified to pH 2.0 with dilute H_2SO_4 (10%), and without separating off of some appearing precipitate, treated with ether for 24 hours in a continuous extractor to eliminate acetic acid.

Neutralized with the saturated $Ba(OH)_2$ and centrifuged. The centrifugate ($BaSO_4$) was washed with 10 cc. of water and the supernatant fluid and washing were united, acidified to pH 4.0 with 10% HCl and distilled in vacuo almost to dryness. The remainder was further evaporated in a like manner three times with 10 cc. of absolute alcohol. To the dried-up matter were added 20 cc. of absolute methanol (The substance dissolved), 0.3 gm. of silver acetate and 1 cc. of acetic anhydride and the mixture shaken for 8 hours (room temperature, 10°C) on a machine and filtered. The bulk on the filter was washed with 50 cc. of hot methanol. The filtrate and washing were distilled together to about 10 cc., and after neutralization with the saturated solution of baryta, concentrated furtheron to dryness. The present residue was dissolved in 10 cc. of water, 10% H_2SO_4 added with precaution to pH 2.0 and a current of ether passed[9] for 24 hours. Neutralization with baryta and centrifugation followed. The centrifugate ($BaSO_4$) was washed with 5 cc. of water and the supernatant liquid distilled together with the washing by vacuum distillation. Two reevaporations to dryness were made, using 20 cc. of absolute methanol each time, and the remaining substance was exhausted twice with 20 cc. of boiling methanol (under reflux). The extracts were separated, when cold, by filtration, united and distilled in vacuo. The substance obtained consisted of a large portion of little granules and a small quantity of mucilaginous substance and amounted to 0.78 gm. (74.5% of the theoretical value).

Since the product was non-homogeneous, fractionation was executed in the following manner. 0.71 gm. of it was taken up in 10 cc. of water, a small insoluble part filtered off and the filtrate distilled under diminished pressure. The yellowish syrup obtained was dissolved in 5 cc. of methanol[10] and 20 cc. of absolute alcohol were added. The somewhat turbid mixture was submitted as such to vacuum distillation, and the residue here dissolved again in 5 cc. of methanol. Addition of 10 cc. of absolute alcohol and 30 cc. of ether gave flocculent precipitates, which were centrifuged off. The clear supernatant fluid was evaporated as above and dried in a vacuum desiccator (H_2SO_4)—0.66 gm. Dissolution in methanol, addition of alcohol and ether and distillation after removal of precipitates, though few, was repeated just like above. The substance given was next dissolved in 3 cc. of methanol, precipitated with 20 cc. of absolute alcohol and 200 cc. of dry ether and the voluminous precipitates centrifuged and dried in a vacuum desiccator (H_2SO_4)—0.57 gm. In this way the mucilaginous part could be almost eliminated but not perfectly. Therefore this product was further subdivided, namely: 0.4 gm. of it was taken up in 3 cc. of methanol and 5 cc. of absolute alcohol and 50 cc. of dry ether added. The deposit separated and dried (0.26 gm.) were again dissolved in 3 cc. of methanol and after mixing of 3 cc. of absolute alcohol and 40 cc. of pe-

troleum ether, stood overnight. The separating upper layer (petroleum ether) was decanted off and the lower layer distilled in vacuo. On the residue were pursued the operations over again employing the organic solvents in volumes equal to the above, and the fraction obtained was dissolved in 3 cc. of methanol again and precipitated with 3 cc. of absolute alcohol and 30 cc. of dry ether. The precipitates (flakes) were centrifuged and dried in a vacuum desiccator (P_2O_5). Homogeneous powder (hygroscopic, non-colored, non-crystalline) free from the glutinous material was obtained. Yield, 0.18 gm.

N (micro Kjeldahl) 3.60% (calc., 3.65%), galactose 47.12% (46.98% by the indole method, 47.26% by the orcinol method) (calc., 47.00%), acetyl 11.18% (calc., 11.23%), iodine use (Macleod and Robison) as glucose 49.50% (calc., 47.00%), ash (micro analysis) 0.53%, rotation in water $\left([\alpha]_D^{20}\right)=\frac{1.123\times 100}{1\times 2.005}=+56.01°$(no mutarotation).

It began swelling at about 95° (maximum swelling at about 135°C) and charring at about 150°C and melted at about 170°–185°C (decomp.), which was similar to the behaviour of the natural disaccharide described in First and Second Paper. It gave the positive indirect-Ehrlich aldehyde (Osaki-Turumi procedure) and Molisch reactions. No anti-isohemoagglutinative potency was observed.

Through the National Research Council was given a grant from the Education Department in aid to us, which is gratefully acknowledged. H. Masamune.

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1) Yosizawa, Tohoku J. Exp. Med., this volume, p. 111.
2) Zemplén, Ber. dtsch. chem. Ges., 1926, **59**, 2402.
3) Fischer and Leuchs, Ber. dtsch. chem. Ges., 1903, **36**, 24.
4) Bergmann and Stern, Chem. Zbl., 1928, **1**, 2663.
5) Zuckerkandl and Messiner-Klebermass, Bioch. Zeits., 1931, **236**, 21.
6) Cf. Levene, J. Biol. Chem., 1916, **26**, 155; Bioch. Z., 1921, **124**, 37.
7) Violent gas evolution was observed. A part of the substance remained still undissolved.
8) Weighed 2.1 gm. when dried in a vacuum desiccator.
9) The precipitates, that had come out by addition of H_2SO_4, were not removed in advance.
10) All the substance dissolved.

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Biochemical Studies on Carbohydrates.

CXXVIII. Structural Study of A-specific Carbohydrate from Mucus Mucin of Pig Stomach.

Fourth Paper.

By

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(Received for publication, May 23, 1949)

How are molecules of the unit disaccharide (N-acetylglucosamine (1,5)-4-*β*-*d*-galactoside(1,5) are linked mutually in the A group carbohydrate? To dissolve this problem, the writer resorted again to the measurement of periodic acid consumption. The materials adapted were the carbohydrate (I) and a partial-degradation product (II) of it obtained by treatment with 6 n. HCl at a room temperature. Calculated from iodine values and sulfate-rest content, their molecular weights amounted approximately to 3600 and 2500 and further analyses pointed to the presence of 9 and 6 molecules respectively of the disaccharide in one molecule of them. And they reduced 10.99 and 8.10 mols of HIO_4 per mol.

Since the substances gave the Ehrlich aldehyde reaction after short heating with weak alkali (Osaki-Turumi procedure), acetylglucosamine stands at one end of the proper-polysaccharide moiety with carbonyl unbound or as linked in a very unstable way. Accordingly, as assumed as a straight chain, the proper-polysaccharides per se should reduce periodic acid per mol as:

Carbohydrate	Mols of periodic acid, when 1-C of acetylglucosamine in one unit disaccharide combines with galactose of the neighbouring disaccharide at			
	2-C	3-C	4-C	6-C
I	11	3	11	19
II	8	3	8	13

Here the terminal monosaccharides, acetylglucosamine and galactose, in a chain consume 3 mols of periodic acid altogether, as is obvious from what has been cited in the second paper.[1] It is seen that the values actually obtained agree quite accurately with the theoretical when 2- or 4-C

of the intermediate galactose molecules takes part in the union between the adjascent untis. Not only the group carbohydrate but also the degradation product possessed an unknown nitrogenous compound and sulfuric acid as further constituents,[2] as already mentioned. But, judging from the findings of the periodic acid use, these non-sugar rests apparently attach to the polysaccharide chain at the positions which are indifferent to the reaction concerned. The amount used of periodic acid is decreased as below, varying with the allocation of the above rests:

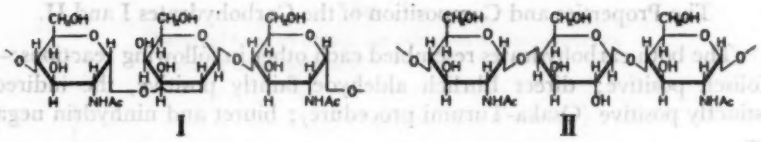
Sugar		Position of attachment	Decrease of periodic acid reduction (mol)
Acetylglucosamine	Terminal	3–C	0
		6–C	1
		3–C, 6–C*	1
	Intermediate	3–C	0
		6–C	0
		3–C, 6–C*	0
Galactose (Terminal and intermediate)		2–C	1
		3–C	2
		4–C	1
		6–C	0
		2–C, 3–C*	2
		2–C, 4–C*	2
		2–C, 6–C*	1
		3–C, 4–C*	2
		3–C, 6–C*	2
		4–C, 6–C*	1

* Cases where the two rests are attached to one and the same sugar.

Namely it looks that the residues are linked at two of the positions 3 of the terminal acetylglucosamine, 3 and 6 of intermediate acetylglucosamine molecules and 6 of galactose molecules, although, if the non-glucosamine nitrogenous component has a reducing force and exactly compensate the decrease of the periodic acid consumption due to its attachment, it must be otherwise alloted.

That 3- or 6-C of intermediate galactose is the position of union between the disaccharide units is least probable, because, on one hand, the reducing force of the non-glucosamine nitrogenous component is hardly estimated so strong as corresponds to 8 (11—3) or 5 (8—3) mols of periodic acid and on the other, wherever the non-sugar residues may be situated, the number of mols of periodic acid reduced can not be lessened to 11 or 8 respectively from 19 (in case of I) or 13 (in case of II). (The maximum decrease by the attachment of the residues is 3).

In consequence, the proper-polysaccharide in the present A group carbohydrate is concluded to have the structure expressed by one of the two diagrams:



I II

That a-from of glucosaminide is assiged is arbitrary. Some of the glucosamine molecules take part in forming the direct-Osaki-Turumi reaction-giving nucleus.[4]

Experimental.

Preparation of the Group Carbohydrate (I).

It was pursued by the method described in the first paper.[3]

Preparation of Carbohydrate II, a Degradation Product of the Group Carbohydrate.

2 gm. of the group carbohydrate (I) were dissolved in 50 cc. of 6 n. HCl and stood in air (room temperature, 20°C). The solution changed brown gradually. After 40 hours, 10 volumes of 94% alcohol and 5 volumes of ether were added and the appearing flocculent precipitates centrifuged, washed twice with 10 cc. of alcohol and dried in a vacuum desiccator (H_2SO_4). A white powder given amounted to 1.4 gm. In the next, its brownish solution in 70 cc. of water was submitted to electrodialysis in a Pauli apparatus (160–200 volts) until renewed water in the outer compartments showed no shift of pH, which took 3 days. Some deposit that came out was filtered off and the filtrate was distilled in vacuo to a small volume at first, then three times to dryness with 20 cc. of absolute alcohol and finally placed in a vacuum desiccator. Yield, 1.2 gm.

Table I.

Analysis	Carbohydrate I %	Carbohydrate II %
Nitrogen*	3.67	3.78
Glucosamine	43.27	41.90
Galactose	43.61	42.16
Indole r.	44.31	42.43
Orinol r.	42.91	41.88
Hydrolysable sulfur	0.95	1.14
Acetyl	10.50	10.15
Ash†	0.32	0.20
Iodine value‡	5.87	8.46

* Micro Kjeldahl. † Micro analysis. ‡ cc. of 0.1 n. iodine solution used by 1 gm. of the carbohydrates. Calculated out from the iodine use by Macleod and Robison method.

The Properties and Composition of the Carbohydrates I and II.

The both carbohydrates resembled each other in following reactions:— Molisch positive; direct Ehrlich aldehyde faintly positive, the indirect distinctly positive (Osaka-Turumi procedure); biuret and ninhydrin negative.

Quantitative-analytical figures obtained are embodied in Table I.

Optical rotation in water.

Carbohydrate I: $[\alpha]_D^{20}=\frac{-0.508\times 100}{1\times 1.92}=-26.46°$

Carbohydrate II: $[\alpha]_D^{20}=\frac{0.094\times 100}{1\times 1.28}=+7.34°$

Molecular weight.

Carbohydrate I: From the sulfur content, $32\times\frac{100}{0.95}=3370$. From the iodine value of it and that of the unit acetylglucosamine galactoside (m. w. 383, iodine value 55.26[5]), $\frac{55.26\times 383}{5.87}=3606$.

Carbohydrate II: From the iodine value, $\frac{55.26\times 383}{8.46}=2502$.

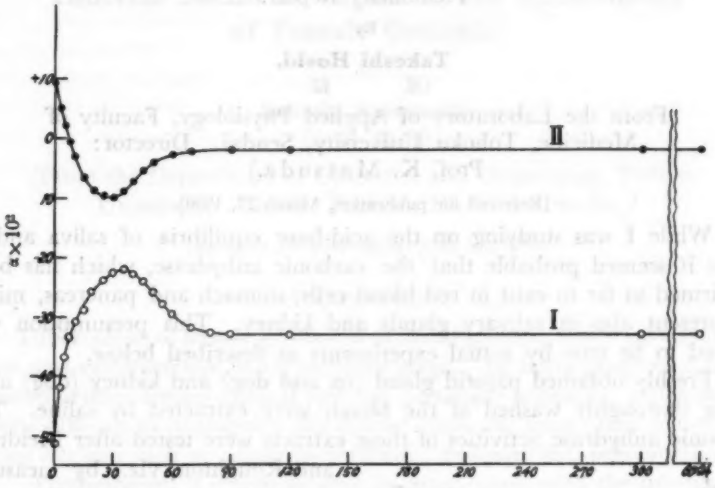
Serological activity.

By our usual procedure, I was found to specifically inhibit the isoagglutination of A-erythrocytes at a dilution of $1:10^6$, but II only at a dilution of $1:10^3$.

Measurement of Periodic Acid Reduction by the Carbohydrates.

The measurement was carried out in a like manner as described in the second paper[1]:— To 5 cc. each of 3.84% solutions of the carbohydrates I and II were added respectively 5 cc. of 0.4364 mol. solution and 10 cc. of 0.1402 mol. solution of periodic acid. Temperature, 20°C. In the both mixtures, change of rotation ceased after 90 minutes from the commencement of reaction (Fig. 1), when 2 cc. of the former mixture and 3 cc. of the latter were pipetted out for determining the acid remaining unreduced. The quantities of the acid reduced reached $0.1172\times\frac{5}{1000}$ and $0.1244\times\frac{5}{1000}$ mol, and accordingly those reduced per mol by the carbohydrates are calculated at 10.99 and 8.10 mols.

Fig. 1.
Change of rotation during the oxidation of Carbohydrates I and II by periodic acid.



Conclusion.

The extents of reducing periodic acid of the A group carbohydrate and a partial degradation product of it suggested that, in the carbohydrate chain, neighbouring two disaccharide units link together through 2- or 4-C of galactose of one molecule and 1-C of accetylglucosamine of the other.

Through the National Research Council was given a grant from the Educational Department for defraying the costs of this work, which is gratefully acknowledged. H. Masamune.

Bibliography and Notes.

1) Yosizawa, Tohoku J. Exp. Med., this volume, p. 111.
2) The readers are referred also to Sinokawa (Tohoku J. Exp. Med., 1949, **50**, 167) and others in this series of investigation.
3) Yosizawa, Tohoku J. Exp. Med., 1949, **51**, 51.
4) It is because the carbohydrate gives the direct Ehrlich reaction. Regarding the structure of this nucleus cf. Hiyama, Tohoku J. Exp. Med., 1949, **51**, 319.
5) Cf. 3).

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Presence of Carbonic Anhydrase in Salivary Glands and Kidney.

Preliminary Report.

By

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(星　　猛)

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(Received for publication, March 27, 1950)

While I was studying on the acid-base equilibria of saliva and of urine it seemed probable that the carbonic anhydrase, which has been confirmed so far to exist in red blood cells, stomach and pancreas, might be present also in salivary glands and kidney. This presumption was proved to be true by actual experiments as described below.

Freshly obtained parotid gland (ox and dog) and kidney (dog) after being thoroughly washed of the blood, were extracted by saline. The carbonic anhydrase activities of these extracts were tested after Meldrum and Roughton, viz., by measurement of accelerating effects for evolution of carbon dioxide from a mixture of phosphate buffer (pH =6.8) and sodium bicarbonate solution.

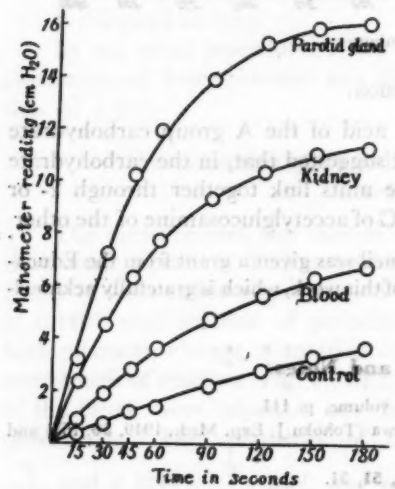

Fig. 1. Carbonic anhydrase activities of parotid gland and kidney as compared with those of whole blood. Effects of extracts of 2 mg wet tissues or blood of dog. Parotid gland and blood of ox have nearly the same activities as dog's respectively.

Typical examples of the results obtained are shown in Fig. 1. Both parotid gland and kidney extracts exhibited intense accelerating activities, which were completely inhibited by 0.002 N KCN. By extract of submental gland of a dog, similarly marked but somewhat weaker effects than by parotid extract were observed. Extracts of brain or muscles tested for purpose of reference showed no such activities at all.

Detailed report as well as its discussion will appear as a forthcoming communication in this Journal.

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Tubercle Bacilli in Vagina in the Tuberculosis of Female Genitals.

By

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(Received for publication, May 30, 1949)

Introduction.

Tuberculosis of female genitals is of latent growth, and it involves only tubes and endometrium at its incipient stage. In fairly progressed cases, it shows neither serious clinical sign nor particular symptom, which makes it very difficult to distinguish this disease clinically from other non-tuberculous diseases such as salpingo-oophoritis, endometritis, pelveoperitonitis etc. Furthermore, it is complicated in many cases with the tuberculosis of other organs, the symptoms of which overshadow its own ones and are liable to make us overlook it. Owing to these reasons stated, the research of the genital tuberculosis has been remarkable slow in advance compared with that in other fields of tuberculosis.

Apart from the hitherto used diagostic methods, in which the biopsy of endometrium and microscopic examination are availed, Professor Dr. T. Shinoda, in collaboration with M. Iwabuchi[1] succeeded in 1939 to make a selective culture of tubercle bacilli from vaginal discharge, menstrual blood and biopsy pieces of endometrium, and thus opened a new realm in the reliable diagnosis of this disease. Then, in collaboration with N. Takahashi,[2] he gave one more advancing step applying the hysterosalpingography and cultivation altogether. Later G. Moriya,[3] examining specific X-ray pictures of tuberculous salpingitis histologically, gave a new significance to the interpretation of X-ray pictures. N. Takahashi, S. Sasamori,[4] and S. Furuhashi[5] researched its connection to the past history and present clinical signs of the tuberculosis of other organs and investigated the clinical process of this disease. Thus its early diagnosis has made a new development, and the whole aspect of this desease has gradually become clearer.

However, as regards the cultivation of tubercle bacilli from vaginal contents, they did not consider its connection to menstruation since M.

Iwabuchi, so the test was positive on the rate of only 75%, and in the rest 25% cases it was possible to turn negative, despite the acutally existing tuberculosis of genitals.

In the tuberculosis of endometrium, which is generally considered to occupy 70–80% of this disease, we can easily suppose that its focus is influenced by the periodical changes of endometrium in menstural period, and the tubercle bacilli which appear in vagina fluctuate in number, and it is supposed that they should exist in the menstrual blood in the largest number, Besides, it can be thought that in the tuberculous salpingitis, which occupies 90–95% of this disease, the focus is influenced to a certain degree by the menstrual period and the number of the appearing tubercle bacilli varies thereby.

So, apart from the aim of raising the positive rate by the cultivation method concerning the diagnosis of tuberculosis, the author tried to find out the very kind and grade of tuberculosis which issues tubercle bacilli in vagina in the largest amount and the term of such occurrence. As regards 4 cases, which were accompanied evidently by tumors, and 24 cases, which were supposed to belong to the incipiency containing comparatively small pains and slight affections,—both of them being chosen from patients diagnosed or suspected to be affected by the tuberculosis of genitalia by the use of many diagnostic methods,—the author researched their vaginal contents and menstrual bloods by means of cultivation, repeating the examination as many times as possible.

Experimental.

Method of Examination.

As the medium for the primary cultures of tubercle bacilli, Oka-Katakura's phosphate—ajinomoto—egg—slants[6] are used, and for the purpose of sterilizing saprophites, 5% H_2SO_4 solution, mingled with Isravin (3.6—diamino—10—methylacridium chloride) at the rate of 0.02%, is availed.

(1) Vaginal Contents: By the help of a vaginal speculum, the contents reservoiring in or adhering to the vagina are taken out with a teaspoon into a sterilized centrifugal-sedimentation-tube as much as possible. About 5–10 time volume of 0.02% isravin mingled with 5% H_2SO_4,[7-8] is still in room temperature for 10 minutes and after being centrifugated for 5 minutes by 3,000 rotation, two platinum eyes of its sediment are spread on the medium and cultivated at 37°C temperature, being sealed with wax. Two egg-slants are used at every examination.

(2) Menstrual blood: By the use of a speculum, menstrual blood and pieces of endometrium remaining in vagina are taken by washing out with sterilized 0.01% Saponin—0.25% $CaCl_2$ solution 18 cc. The sub-

stance thus obtained is left still in a ice-room for an hour, then centrifugated for 5 minutes by 3,000 rotation. Its sediment is then mingled with 5 cc. of sterile water, smashed in a mortar, and again centrifugated for 5 minutes. To the sediment thus obtaind 5–10 time volume of solution containing 10% saccharated pepsin, 0.02% Isravin and 5% H_2SO_4 is added. After the interval of 10 minutes, this is centrifugated for 5 minutes, and the sediment thus obtained is cultivated by the above-mentioned method.

(3) The way of discrimination is as follows: three weeks after the beginning of cultivation, opaque creamy-white dry colonies, resembling wax in shape, begin to appear on the medium to be recognized by the naked eye. The Ziehl-Nielsen's staining test proves acid-alcohol-fast bacilli and the Kaufmann-Preis' boiling test[9-10], shows more than 3 minutes. In such instance. It is concluded that the existence of bacilli has been proved (See Table I).

Result of the Examination.

The early stage of the tuberculosis of female genitals is not accompanied by an abnormal menstruation, but in cases advanced to a certain degree, as widely known, there often occur abnormal menstruation, i.e. oligomenorrhea, hypomenorrhea occurred in 4, oligomenorrhea in 6, and in the other 18 cases menses were almost of no abnormality. In these 24 cases, except amenorrhea, no remarkable change were recognized in the affections or functions of uteri and ovaries.

18 *cases accompanied by normal menstruation.*

In the above 18 cases of normal menses, it was possible to prove bacilli from 5 days before menses through the whole menstrual period; especially in the discharge 1–2 days, they appeared in the largest number. In 10 cases of them a few number of bacilli were recognized in the remaining menstrual term, which, in the other 8 cases, was impossible to take place except in the premenstrual days and the menstrual term; those belonged to cases of slight degree, in which comparatively primary or slight affection existed in tubes without causing menstrual abnormality. This fact is very valuable for the secure diagnosis of slight degree, in wihch wholly or almost wholly no clinical change can be recognized.

6 *cases of oligomenorrhea.*

In one case among the 6 cases of oligomenorrhea stated, the endometrium revealed remarkable tuberculous affection; amenorrhea was about to occurr; and tubercle bacilli were always evidenced in a large amount. However, the spontaneous rupture of the salpingeal abscess has brought

the present condition of a brief tranquility. While in the rest 3 cases, though many bacilli were being issued abundantly in the 1–2 premenstrual days and primary term of menses, bacilli were almost absent or few if any during the intermediate term. Especially in the cases 19. and 20. endometrial pieces revealed no affection histologically, and only tuberculous salpingitis existed: the general condition was benign and bacilli were evidenced in a minimal number. In the cases 21. and 22, tuberculous afection were evidenced in the tubes and the mucosae of the uterus. In the latter, especially, a big abscess was present, which occupied the greatest part of the pelvic cavity and bacilli were also issued in the largest number. In the case 23, no histological examination of the endometrium was tried, but afterwards she was operated for bilateral salpingeal abscess, and a few bacilli were confirmed their appearance.

According to the investigation of the processes of those cases during the following year, no death has been reported. In two cases, 20. and 23, it seems that self-conscious sysmptoms have somewhat increased, while in the other cases rather decreased. However they are not recovered by any means: they are now in chronic condition.

4 *cases of amenorrhea* (see Table I).

In one case (case 28), among 4 cases of amenorrheal genital tuberculosis, the patient have been amenorrheal and infertile for three years: both tuberculous chronic adnexitis and endometritis have turned neither better nor worse during three years, a little amount of tubercle bacilli continually appearing. In such case as this, which is often seen by other genital tuberculosis with menses, despite of comparatively advanced endometrial affection, the process is generally favorable, but the affection of tubes makes no advance and tuberculous affections of other organs are recovered owing to a good nourishment. But as long as tubercle bacilli appear, however small they may be in number, the patient can not be called completely recovered.

In the other 3 cases, 6–8 para, affection occurred in lungs or peritoneum and the disease took place almost simultaneously with it. Remarkable tuberculous affection was recognized also in the endometrial tissues. In every one of them bacilli appear abundantly. And they died several months thereafter from progressed plumonary tuberculosis, tuberculous peritonitis or intestinal tuberculosis. Considering these facts, it should be concluded that the prognosis is malignant when amenorrhea follows after genital tuberculosis and abundant bacilli appear especially in such case that other organs are complicated apparently by active affections.

Case Number	Age	Years married	Number pregnancy	Chief complains	Tub ana
1	29	5	0	sterility abdominal pain	unconsc
2	31	3	0	sterility abdominal pain	26. sero
3	24	3	0	sterility	18. sero 19. iriti
4	33	11	0	sterility leukorrhea	18. dry
5	27	4	0	sterility	19. sero 25. peri
6	32	7	0	sterility menorrhagia	18. peri
7	24	2	0	abdominal strain	24. sero
8	28	5	0	sterility	19. pleu toni 27. hilu
9	36	17	0	sterility leukorrhea	unconsc
10	28	10	0	sterility	unconsc
11	30	4	0	sterility	25. sero
12	30	8	2	abdominal pain	22. Pulr
13	25			leukorrhea menorrhagia	23. sero
14	23	4	0	sterility leukorrhea	16. Pulr
15	20	2	0	sterility leukorrhea	15. sero
16	32	7	0	sterility abdominal pain	25. Pyo 31. peri
17	27	6	0	sterility	15. peri
18	31	10	0	sterility	24. dry
19	26	3	1	abdominal pain leukorrhea	24. sero 25. pleu
20	24	4	0	sterility abdonimal pain	14. sero
21	25	2	0	abdominal pain oligomenorrhea	18. peri

TABLE I.

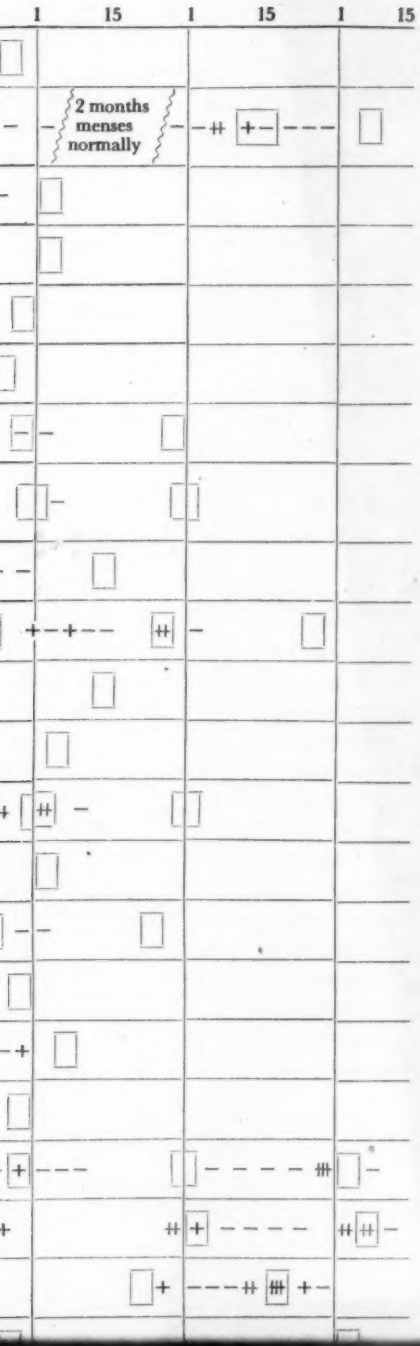
	Tuberculous* anamnesis	Clinical† diagnosis	Physical examination	Microscop. examination (tbc)‖‖	Results of cultivation: 1. by naked eye + 1~4, 2. □ indicate Date 1 15 1 15 1 15
pain	unconscious	tuberculous adnexitis‡	nothing revealed		+ □+ +++#[+] + --
pain	26. serous pleurisy	tbc adnexitis§	tuberculous peritonitis	E(−) T(+)	□+ + −− [+] − − − − □ −
	18. serous pleurisy 19. iritis (tbc)	tbc salpingitis	nothing revealed	E(+)	□ − +[+]+ −
	18. dry pleurisy	tbc salpingitis	„		□ −+[+]−−−
	19. serous pleurisy 25. peritonitis	adnexitis pelveoperitonitis	„	E(−)	□ −−[+]− − −
ia	18. peritonitis	tbc adnexitis	„	E(−)	□ [+]+ − −
	24. serous pleurisy	ovarian tumor	pleurisy healed	E(+)	+ □ −− □ −−+−
	19. pleuroperitonitis 27. hilus gland tbc	tbc adnexitis	strongly marked hilus shadows	E(−)	□ −−− #[+] −−−−
	unconscious	metroendometritis‖	left hilus tbc	E(−) T(+)	□ −− [+] −+ − # [#]
	unconscious	adnexitis	pulmonary tbc (calcified)	E(+)	# [#] + # □ −#
	25. serous pleurisy	tbc adnexitis	nothing revealed	E(−)	□ − −−+[+]−− −− [+]
pain	22. Pulmonary tbc	pelveoperitoitis	pulm. tbc.	E(−)	□ −− +[−] −−
a	23. serous pleurisy	pelveoperitoitis	nothing revealed		□ −−+#[+]□ − − −
	16. Pulmonary tbc	tbc salpingitis	„	E(−)	□ −+−−+[−]−−
	15. serous pleurisy	tbc adnexitis	left pulm. infiltration	E(−)	□ − [+] −−
pain	25. Pyothorax 31. peritonitis	adnexitis¶	intestinal tbc?	E(−)	□ −−−+[−] −−−−
	15. peritonitis	tbc adnexitis**	nothing revealed	E(−) T(+)	□ − + + # [#]
	24. dry pleurisy	cervix catarrh	„		□ −−− #[+]−−−−
ain	24. serous pleurisy 25. pleuroperitonitis	tbc adnexitis	tubercular mass in mesentery	E(−)	[+] −+−−− −−−−
ain	14. serous pleurisy	endometritis adnexitis††	strong hilus shadows on both sides	E(−) T(+)	□ − − −
ain hea	18. peritonitis	pelveoperitonitis tbc adnexitis	nothing revealed	E(+)	□ # +−++ − − −

TABLE I.

	Physical examination	Microscop. examination (tbc)‖‖	Results of cultivation: 1. by naked eye computable colony + 1~4, ++ 5~10, ## 2. [ ] indicates menses. Date 1 15 1 15 1 15 1 15
	nothing revealed		+ [+] + + + + ## [+] + -- [ ]
	tuberculous peritonitis	E(−) T(+)	[ ] + + -- [+] - - - - [ ] - - - - 2 mont menses normall
	nothing revealed	E(+)	[ ] - + [+] + - - - [ ]
	„		[ ] - + [+] - - - [ ]
	„	E(−)	[ ] - - [+] - - - [ ]
	„	E(−)	[ ] [+] + - - [ ]
	pleurisy healed	E(+)	+ [ ] - - [ ] - - + - + [-] -
	strongly marked hilus shadows	E(−)	[ ] - - - ## [+] - - - - [ ] -
is‖	left hilus tbc	E(−) T(+)	[ ] - - [+] - + - ## [##] + - [ ]
	pulmonary tbc (calcified)	E(+)	## [###] + ## [ ] - ### [##] + - + - -
	nothing revealed	E(−)	[ ] - - - + [+] - - - - [+] - [ ]
	pulm. tbc.	E(−)	[ ] - - + [-] - - - [ ]
	nothing revealed		[ ] - - + + ## [+] [ ] - - - + ## [##] -
	„	E(−)	[ ] - + - - + [-] - - [ ]
	left pulm. infiltration	E(−)	[ ] - [+] - - + [-] - -
	intestinal tbc?	E(−)	[ ] - - - + [-] - - - [ ]
	nothing revealed	E(−) T(+)	[ ] - + + ## [##] + + - + [ ]
	„		[ ] - - - ## [+] - - - - [ ]
	tubercular mass in mesentery	E(−)	[+] - + - - - - - - - + + [+] - - -
	strong hilus shadows on both sides	E(−) T(+)	[ ] - - - [##] +
	nothing revealed	E(+)	[ ] ## + - + + - - -

6 | 32 | 7 | 0 | sterility menorrhagia | 18. periton

mputable colony-number of tubercle bacillus
+ 5~10, ++ 11~50, +++ over 50
nenses.



	18. peritonitis	tbc adnexitis	„	E (−)			+ + − −

	nothing revealed	E (+)		++	+−++	− − −	

6	32	7	0	sterility menorrhagia	18. peri
7	24	2	0	abdominal strain	24. sero
8	28	5	0	sterility	19. pleu toni 27. hilu
9	36	17	0	sterility leukorrhea	unconsc
10	28	10	0	sterility	unconsci
11	30	4	0	sterility	25. serou
12	30	8	2	abdominal pain	22. Puln
13	25	/	/	leukorrhea menorrhagia	23. serou
14	23	4	0	sterility leukorrhea	16. Puln
15	20	2	0	sterility leukorrhea	15. serou
16	32	7	0	sterility abdominal pain	25. Pyot 31. perit
17	27	6	0	sterility	15. perit
18	31	10	0	sterility	24. dry p
19	26	3	1	abdominal pain leukorrhea	24. serou 25. pleur
20	24	4	0	sterility abdonimal pain	14. serou
21	25	2	0	abdominal pain oligomenorrhea	18. perit
22	23	/	/	abdominal pain oligomenorrhea	20. pleur
23	24	5	0	sterility leukorrhea	16. pulm
24	31	8	4	abdominal pain	30. serou 31. perit
25	39	20	6	leukorrhea amenorrhea	38. pulm
26	38	16	6	abdominal pain	unconscio
27	45	18	8	abdomidal pain leukorrhea	32. serou
28	30	3	0	amenorrhea sterility	22. pleur

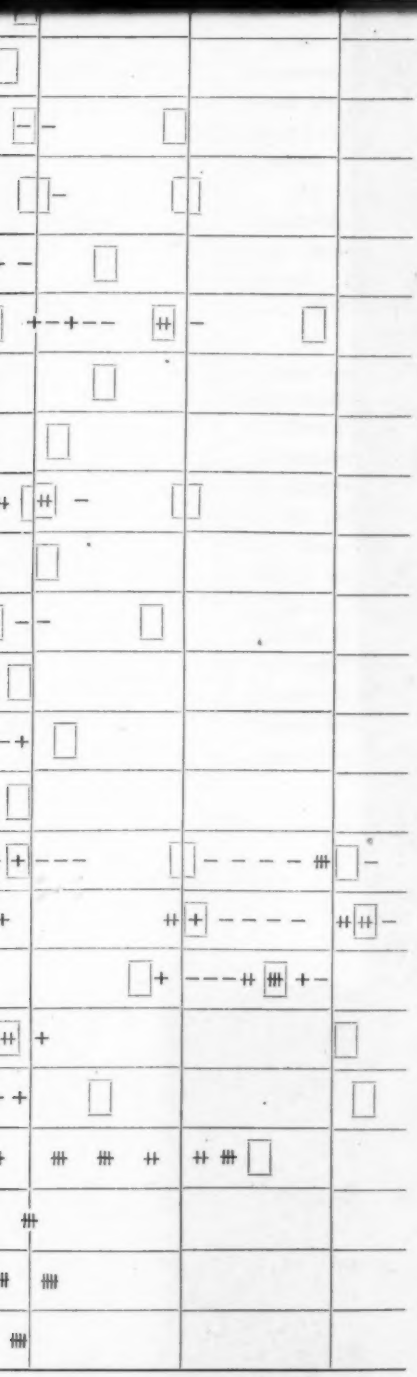
* Number under tuberculous anamnesis indicates ye
and tbc peritonitis. ‖ tbc endosalpingitis. ¶ Int
endometritis. §§ tbc pelveoperitonitis. ‖‖ The d
stance. E: endometrium, P: portio, T: tube.

ia	18. peritonitis	tbc adnexitis	„	E (−)				
	24. serous pleurisy	ovarian tumor	pleurisy healed	E (+)				
	19. pleuroperitonitis 27. hilus gland tbc	tbc adnexitis	strongly marked hilus shadows	E (−)				
	unconscious	metroendometritis‖	left hilus tbc	E (−) T (+)				
	unconscious	adnexitis	pulmonary tbc (calcified)	E (+)				
	25. serous pleurisy	tbc adnexitis	nothing revealed	E (−)				
ain	22. Pulmonary tbc	pelveoperitoitis	pulm. tbc.	E (−)				
a	23. serous pleurisy	pelveoperitoitis	nothing revealed					
	16. Pulmonary tbc	tbc salpingitis	„	E (−)				
	15. serous pleurisy	tbc adnexitis	left pulm. infiltration	E (−)				
ain	25. Pyothorax 31. peritonitis	adnexitis¶	intestinal tbc?	E (−)				
	15. peritonitis	tbc adnexitis**	nothing revealed	E (−) T (+)				
	24. dry pleurisy	cervix catarrh	„					
ain	24. serous pleurisy 25. pleuroperitonitis	tbc adnexitis	tubercular mass in mesentery	E (−)				
ain	14. serous pleurisy	endometritis adnexitis††	strong hilus shadows on both sides	E (−) T (+)				
ain hea	18. peritonitis	pelveoperitonitis tbc adnexitis	nothing revealed	E (+)				
ain hea	20. pleuroperitonitis	adnexal tumor (tbc)‡‡	pulm. tbc	T (+)				
	16. pulmonary tbc	gonorrheal adnexitis endometritis	left apex infiltration					
ain	30. serous pleurisy 31. peritonitis	Douglas abscess	pulm. tbc. (calcified)	E (++)				
	38. pulmonary tbc	endometritis erosion (tbc)	pulm. tbc	¶¶				
ain	unconscious	adnexal tumor (tubal hematom?)§§	postoperative pleurisy	¶¶				
ain	32. serous pleurisy	portio-carcinom?	pulm. tbc	P (++)				
	22. pleuroperinitis	tbc adnexitis	nothing revealed	E (+)	since (November 1942 amenorrhea) 26/V, 1943 16/VI 16/VII 21/II, 19 + + + −			

esis indicates years of age. † The designation "‡∼§§" indicates the postoperative diagnosis. ‡ Tuberculous salp
pingitis. ¶ Intestne-istenosis caused by adhesion owing to tbc peritonitis. ** tbc pyosalpinx and pelveoperitonitis.
tis. ‖‖ The designation (+) indicates the tuberculous affection, and (−) negative. "¶¶" indicates that the biop
o, T: tube.

	”	E (−)	[ ]	[+] +	− − [ ]	
	pleurisy healed	E (+)	+ [ ]	− − [ ]	− − + − + [−]	−
	strongly marked hilus shadows	E (−)	[ ]	− − − ++ [+]	− − − − [ ]	−
is‖	left hilus tbc	E (−) T (+)	[ ]	− − [+] − +	− ++ [++] + −	[ ]
	pulmonary tbc (calcified)	E (+)	++ [+++]	+ ++ [ ]	− +++ [++] +	− + − −
	nothing revealed	E (−)	[ ] −	− − + [+] − −	− − [+] −	[ ]
	pulm. tbc.	E (−)		[ ] − −	+ [−] − − −	[ ]
	nothing revealed		[ ]	− − + + ++ [+]	− − − − + ++ [ ]	[++] −
	”	E (−)	[ ]	− + − − +	[−] − −	[ ]
	left pulm. infiltration	E (−)	[ ]	− [+] −	− + [−] −	−
	intestinal tbc?	E (−)	[ ]	− − − + [−]	− − − [ ]	
	nothing revealed	E (−) T (+)		[ ] − + +	++ [++] + + − +	[ ]
	”		[ ]	− − − ++ [+]	− − − − [ ]	
	tubercular mass in mesentery	E (−)	[+]	− + − − −	− − − − + + [+]	− − −
	strong hilus shadows on both sides	E (−) T (+)	[ ]	− −	− [++] +	
	nothing revealed	E (+)	[ ]	++ + − + +	− − −	[ ]
	pulm. tbc	T (+)	[ ] − +	[+]	+ + [++]	+
xi-	left apex infiltration		[ ] +	+	+ + [++] + + +	[ ]
	pulm. tbc. (calcified)	E (++)	[ ] +++ +++ [+++]	[+] + +++	+++ +++ +++ +++	+++ +++
	pulm. tbc	¶¶		+++ ++++ ++++	+++ ++++	+++
	postoperative pleurisy	¶¶		+++ +++	+++ +++ ++++	++++
)§§						
?	pulm. tbc	P (++)		++++ +++	++++ +++ ++++	
	nothing revealed	E (+)	since (November 1942 amenorrhea) 26/V, 1943 16/VI 16/VII 21/II, 1944 1/V 12/VI 14/III, + + + − + + +			

n "‡∼§§" indicates the postoperative diagnosis. ‡ Tuberculous salpingitis and hypoplastic
sion owing to tbc peritonitis. ** tbc pyosalpinx and pelveoperitonitis. †† tbc salpingitis.
uberculous affection, and (−) negative. "¶¶" indicates that the biopsy of endometrium rev



12/VI 14/III, 1946 28/V 10/VII 6/IX, 4/XI
+ + + + + +

and hypoplastic uterus. § tbc pyosalpinx
tbc salpingitis. ‡‡ tbc salpingitis and tbc
endometrium revealed caseous necrotic sub-

Summary and Considerations.

With 28 cases of genital tuberculosis which were diagnosed, the author examined vaginal discharge and menstrual blood repeatedly to investigate the prosperity and decay of tubercle bacilli, and got the following consequences.

(1) In 18 cases which were accompanied by normal mentruation, bacilli began to be proved in large number from 5 days before the menstrual term on, and 1–2 days before the menstrual term or on 1st–2nd menstrual days they appeared in the largest number. In the remaining menstrual term bacilli were not proved, or if any very few. In 8 of 18 cases, bacilli were negative in the rest term.

(2) In 5 cases of 6 cases of oligomenorrhea, bacilli appeared in the largest number shortly before or through menstrual term. In the other 1 case, numerous bacilli appeared constantly, but they diminished soon after the menses. In all cases, however, they could be proved, in the rest menstrual term, though not always, in small number.

(3) In 3 out of 4 cases accompanied by the complaint of amenorrhea, numerous tubercle bacilli were always inssued, and these cases were all followed by malignant prognosis. In the other 1 case, more than 3 years elapsed with no remarkable change, bacilli constantly appearing in small number.

(4) In 2 cases, in which necrotic substance was proved by the biopsy of endometrial tissues, and in 2 cases, in which advanced tuberculous affections were recognized microscopically, numerous bacilli were always proved. In a case accompanied by menses (Case 24). bacilli diminished soon after menstruation.

(5) In 4 cases, in which slight tuberculous affections were proved microscopically by the biopsy of endometrial tissue, in 14 cases in which they could not be proved, in 5 cases in which some affections were proved in tubes after the practice of laparotomy, and in the other 5 cases in which no histological examination was tried, tubercle bacilli were all proved in the largest number before menstruation or in the early menstrual term. In the remaining menstrual term, they were entirely negative, or if any, very few. Thus, it was acertained that, to say nothing of these cases in which tuberculous affections were proved in endometrium, in cases of slight or primary tuberculous salpingitis in which the affections were considered to be localized in tubes only, the number of bacilli appearing in the vagina varies according to the menstrual term, they appear evidently in several pre-menstrual days and they can also be proved in menstrual blood, but in the remaining intermediate term they do not appear; or if any, they are very few.

It was easily explained that, when the endometrial tissue was destroyed or excorriated on the 1st-2nd menstrual days, bacilli should appear in the largest number due to the affection of endometrium. It is a matter of deep interest that the number of appearing bacilli increases rapidly from 5 days before menses on, and on the 1st–2nd pre-menstrual days they are found no less than or rather more than those on the 1st–2nd menstrual days, for it accords to the secretive function of endometrium. Especially it gives a secure proof for the diagnosis of salpingeal tuberculosis that bacilli appear in the final term of secretion in such cases in which no tuberculous affection is recognized in the endometrium and in which the affection is considered to be localized in tubes, and in the remaining menstrual term they do not appear at all, or if any, they are very few.

From those facts stated, the availability of the cultivation method for the early diagnosis of genital tuberculosis becomes clearer, and it can be ascertained that the cultivation of vaginal contents on 1st–2nd pre-menstrual days and of menstrual blood on 1st–2nd menstrual days is the most effective in cases of primary of slight genital tuberculosis.

Conclusion.

The author practised selective culture from vaginal contents and menstrual blood repeatedly, and ascertained that, for the purpose of diagnosing its existence, to say nothing of endometrial tuberculosis, even in those cases accompanied by primary genital tuberculosis, in which the affections were considered to be localized in tubes, the culture of vaginal contents on 1st–2nd pre-menstrual days or of menstrual blood on 1st–2nd days of the menstrual term, in which bacilli appear in the largest amount, makes the positive rate the highest and so it is the most effective way.

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Clinical Study of the Toxemia of Late Pregnancy, with Special Reference to Its Recurrent Conditions, Transition to Chronic Type and Eclampsia.

1st Report.

On the Toxemias of Late Pregnancy Excluding Eclampsia.

By

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Pregnancy is orginally physiological. We know, however, that it easily inclines to be abnormal, and is apt to lead to the so-called toxemia of late pregnancy in the latter half, especially after the seventh month of pregnancy. According to our investigation, the toxemia of late pregnancy have been found at the rate of 21.5 per cent of all pregnant women. It is a well-known fact that some of these toxemias cause eclampsia, others lead to various symptoms or complications and threaten both mothers and children with death. But we seldom find the studies concerning how these toxemias affect, the subsequent pregancies, and those results are not always agreeable. There are different views on the problem, whether the toxemia may actually induce chronic nephritis, or not. (i.e.,) (1). Trantenroth,[1] Schultz,[2] Effkemann,[3] Neuweiler u. Scharplaz,[4] Heynemann,[5] Reida. Teel[6] and Theobald[7] are negative. (2). Banister[8] and Kaji[9] are sceptical. (3). Fischer,[10] Gibberd,[11] Kuji,[12] Inada,[13] Fujii,[14] Herricka. Tillmann,[15] Hofmeier,[16] Fujikawa,[17] Fehling,[18] Wolf u. Zade,[19] Peckham,[20] Seitz[21] and Nakayama-Doi[22] are affirmative. However, these discussions are viewed mostly from the clinical sights. And pathologically since Fahr, so called nephropathia gravidarum is always nephrosis and not nephritis. Consequently it is right, as Effkemann says, that nephropathia grav, which develop into chronic course is equal to chronic nephrosis. However, on the other hand they are not always benign, and some pathologists agreed with that so called nephropathia grav, at times lead to nephrosclerosis and then often develop into death. At any rate it is clinically true that some parts of toxemias will make as invasion upon maternal health in future, so it is an important matter for the diagnosis of subsequent course to make comparison with the conditions of subsequent pregnancies and

each type of toxemias.

Therefore, 141 patients of the non-convulsive toxemia of late pregnancy have been successively observed from their first pregnancies and the other 44 cases from their second or third pregnancies through each proscess of pregnancies and progress of childbed, and it has been studied to find the base of judgement of their prognosis.

Classification.

Out of main symptoms we have established the limits of toxemia as follows: Albuminuria—over 0.5‰. Edema at least comes to abdomen. Blood pressure—over 150 mm. Hg. systolic. When these symptoms arise independently, we call them respectively type A, type O, and type H and when they are complicated with each other, type AO, type OH, type AH and type AOH. Initial attack of toxemia which does not turn to chronic toxemia is called primary toxemia. And subsequent attacks which also does not turn to chronic are called secondary toxemia. When toxemia does not disappear even one month later in the stage of post puerperium, we call them chronic toxemia.

Results of Investigations.

Each type of toxemias pregnancy.

TABLE I.

The Distribution of Each Type of Non-convulsion Toxemia (1).

	Toxemia type	A	O	H	AO	OH	AH	AOH	Total
Primary toxemia	Primigravid 91 cases	23.1%	12.1	23.5	12.1	4.4	8.8	15.4	100%
	Multigravid 50 cases	16.0	20.0	22.0	6.0	14.0	16.0	6.0	100
	Whole 141 cases	19.8	15.6	23.4	9.9	7.7	11.3	12.0	100
Secondary toxemia 116 cases		18.9	18.1	37.9	5.1	7.7	6.0	14.9	100
Chronic toxemia 37 cases				33.3		5.3	24.6	36.8	100

Table I shows the distribution of each type of toxemias. If the toxemia attacks both primigravida and multigravida primary, it should be distributed in equal rate in pregnant women, but in the case of multigarvida the distributional rate of type OH, type AH and type O is two or three more, consequently type AOH and type AO are less than $\frac{1}{2}$ in the case of

multipara. And we can not put the primary toxemia of multigravida and mild attack of secondary toxemia in the same category. In short we cannot help recognizing the specific existence of multigravidous primary toxemia.

TABLE II.

The Distribution of Each Type a Number of Pregnancy in Nonconvulsive Primary Toxemia (Cases)

Pregnant number \ Toxemia type	A	O	H	AO	OH	AH	AOH
Primi-grav.	21	11	22	11	4	8	14
bi-grav.	6	6	9	3	5	8	1
tri-grav.	2	1	2		2		2
IV-grav.		3					

Table II shows the relation between number of pregnancies and types of toxemia. The greater part of primary toxemia of multipara appear in bi-gravida. But some part appear in tri- and tetra-gravida, and two thirds of them are accompanied by edema. I wonder if there is any relation between edema and number of pregnancy. Though it is secondary toxemia we have two specific examples which arose in the sixth pregnancy i.e. they were type AOH and type AH, and they disappeared rapidly after delivery, and the seventh pregnancy was normal. So I can not agree with the views of Ogata,[23] Inada,[13] and others, who assert that pregnancy toxemia which arise after the fourth pregnancy is not nephropathia gravidarum.

The relation between the types and outbreak ages of toxemias is as follows: in primary toxemia type A (22.6%) and type H (20.7%) are absolutely many under 25 years of age and next to these type O (15.1%) and type AOH (13.2%) show high rate. Between 25 and 29 years of age type A (24.6%) and type H (24.6%) are found many too, and the rest types are found most in equal rate. However in the case of over 30 years of age, type A (10.5–0%) is relatively few and type O (26.3–50%) and type H (21–5.0%) are high rate and type AOH is next to these. According to this results, it seems to us that in primary toxemia type A is liable to appear in younger primigravida and type O in old cases. In secondary toxemia type H (27–60%) is the highest rate through every age, but it is not parallel with age. Type O (28.8%) is the most in the age between 25 and 29 years of age and in the case over 30 years it is very few (4–5%). This fact differs from the case of primary toxemia. Type AH (0–6.6–20%) and type AOH (9–24–36%) have tendency to increase with age, especially type AOH increases remarkably and these will be due to following fact that with age,

with repeated toxemias, the invasive grade upon maternal bodies gradually grows, affecting other parts of body.

State of recurrence in the toxemia which not turn into chronic.

TABLE III.

Second Attack of Non-convulsive Primary Toxemia (Cases).

Type at second attack \ Type of prim. toxem	A	O	H	AO	OH	AH	AOH	Total
A	6	2		2		1	3	14
O	4	2	3	1		1	1	12
H	2	2	8	1	3	3	5	24
AO		1				1		2
OH	1			1		1		3
AH	1		1			1	1	4
AOH	2	1		1	1	1	2	8
Totol	16	8	12	6	4	9	12	67
normal	12	6	16	8	4	5	5	55
Rate of second attack	57.1%	57.1	42.8	42.8	50.0	64.3	75.0	54.9%

TABLE IV.

Second Attack of Non-convulsive Secondary Toxemia (Cases).

Type at second attack \ Type at second toxemia	A	O	H	AO	OH	AH	AOH	Total
A	4		1	2			1	8
O		2		1				3
H	2		7		3	1	2	15
AO			1		2			3
OH		2	1					3
AH	1		2			1		4
AOH	3		1	1	1	1	2	8
Total	10	4	13	3	6	3	5	44
Normal	4	5	5	2	1	2	2	21
Rate of second attack	71.4	44.7	72.2	60.0	85.7	60.0	71.4	67.7

67 cases out of 122 cases in primary toxemia (54.9%), and 44 cases of 65 cases (67.7%) in secondary toxemia have had appearance of toxemia

at next pregnancy. The rate of second attack of primary toxemia which Young, Sym and Crowe[24] has reported is 55.8%, which coincides ours. Other rates show: Busch,[25] 33%, Bunzel,[26] 45%, Gibberd[27] a. Sym,[28] each 50%. Page and Cox,[29] 65%. Table III and IV show us the rate of second attack of each toxemic type. From the types of toxemia at second attack, we find that they show quite various types, but generally in secondary toxemia they are greatly inclined to type H, but we recognize that they also, for the most part, have had toxemias with hypertension last time. Among the toxemias without hypertension, there are no mutual relation between them and the types of subsequent toxemia, but the rate of second attack among them is far higher than that of toxemia of pregnancy (21.5%) and they are more liable to recur when they have passed toxemia. These facts signify that the difference between each type will not be essential, but a mere difference caused by the fact that the impediment or reaction of whole body against pregnant toxin will appeare strongly in Loccus minor is of each cases. So once she passes toxemia, then she is liable to be influenced by toxemia again next time. About the relation between outbreakage and the rate of second attack, as a whole, concluded with both primary and secondary toxemia, the rate of second attack is elevated with the age. When classified by age, the rate of second attack both in primary and secondary toxemias between 20 and 24 years of age are each 60.3% and 33.3%, between 25 and 29 years of age are each 45.3% and 65.4%, between 30 and 34 years of age are each 72.7% and 80%, and between 35 and 39 years of age in secondary toxemia it is 81.2% and over 40 years of age in 100%. And the rate of second attack is exceedingly different between each type of toxemia. The rate of second attack in subsequent pregnancy arises awful different with the age between primary and secondary toxemia of each type. For example, in type A, the rate of second attack of primary toxemia decreases remarkably (9/12% cases), on the courtary, its rate of secondary toxemia increases with the age (1/3–4/5 cases). On the other hand, in type H, the rate of second attack of primary toxemia does not increase so much even the age grows older, but the rate of second attack of secondary toxemia increase with the age (6/9–3/3 cases). The principal cause which increases the rate of second attack can not be found in case of primary toxemia, but in secondary toxemia it owes to type A, type H, type OH, type AH and type AOH. In short, toxemia with hypertension and type A are liable to recur and type O is not. Table V shows as the situation of outbreak by difference of pregnancy frequency of secondary toxemia. On and after IV- Gravida, (suddenly) they have tendency to have repeated attacks, especially on and after X-gravida all have repeated attacks and all cases on and after IV-gravida of type OH and VII-gravida of type H have repeated attacks. There is no specific relation between

TABLE V.

Frequency of Non-convulsive Recurrent and Chronic Toxemia Separated by Pregnant Order.

Pregnant order	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Recurrence non	35:48	18:27	20:4	8:5	15:3	10:2	3:2	4:1	3:0	1:0	1:0	1:0
Frequency of second attack	42.1	40.0	88.3	61.5	83.3	83.3	60.0	80.0	100.0	100.0	100.0	100.0
Frequency of chronic toxemia out of whole toxemia	7.8	10.5	16.1	18.1	22.2	47.5		48.0			60.0	

delivery interval and rate of second attack, and it does not mean that the longer the delivery interval is, the less the rate of second attack grows. One may recognize that the rate of second attack of the cases over 4 years interval is less than that of the cases under 4 years interval.

About the relation between classes of inpatients and rate of second attack in primary toxemia, the lower class inpatients have a tendency to recur toxemia (1st class 0%, 2nd 43%, 3rd 35%, free 58%), especially in secondary toxemia it is remarkable (1st 0%, 2nd 60%, 3rd 73%, free 71%), in type AOH, type H and type OH. About relation between grade of symptoms and second attack, in primary toxemia they are liable to recur in the cases of over 180 mm Hg. blood pressure or edema of whole body at high grade, but these is no relation with quantity of albuminuria. Among secondary toxemia the rate of second attack is paralleled with grade of these symptom, especially it is liable to recur in the cases of albuminuria over 5% or blood pressure over 170 mm. Hg.

Transition to chronic type.

The rate of transition into the chronic nephritis from pregnancy toxemia is 10% by Gibberd,[27] 28% by Evans,[30] and 40% by Peckham.[20] And there are extreme differences in their rates. In our case, it is 15.6% and from primary toxemia is only 3.5%, but from secondary toxemia it is 7 times more; it shows 24%. It is a striking fact that only 4 types with hypertension, namely type H, type OH, type AH and type AOH have usually become to chronic type, and 3 types which do not take hypertension, namely type A, type O and type AO have not become to chronic. In primary toxemia type AOH at the rate of 17.6% (3/17 cases), type OH 9.1% (1/11) and type H 3.0% (1/33) change to chronic type and we find no other type. However, secondary toxemia, type AH at the rate of 60.8% (14/23), type AOH 45.0% (18/40), type H 27.2% (18/66) and type OH

14.3% (2/14), change to chronic type. That the frequency of chronic type separated by age increases with the age is a clear fact, though in primary toxemia such cases are few. In secondary toxemia it is distinct, especially over 30 years of age it shows 30%, over 40 years of age 42.8% and over 45 years of age it is advanced to 60%. Table V also shown the relation between pregnant order and chronic type. With the higher order, the more frequent in the appearance of chronic type against non-chronic type. And such an elevation of frequency is always shown in type H, type AH and Type AOH which are accompanied with hypertension and seldom in type OH, and type A, type O and type AO do not become chronic type. In consequence, it would be no great error to say that among multigravida a greater part of type H, type AH or type AOH will turn to chronic toxemia. Kuji[31] says that there are many cases of nephropathia gravidarum in multipara which take unfavourable progress against recovery. This will be due to the existence of chronic toxemia. The highest rate of frequency of chronic type, when observed by inpatients class, appears in free patients (21.9%) and as the class is higher, the frequency becomes less (3rd class 13.3% 2nd 8.5%) and there is no chronic patient in first class. Chronic toxemia has always repeated pregnancy toxemia next time and shows various types of toxemias, but generally it has been recurred by toxemic type with hypertension (type H, type AH, type OH and type AOH). When we observe chronic toexemia, among subsequent frequencies, type H takes place at the rate of 11/27 cases, type AOH 9/27 cases, type AH 6/27 cases and type OH 1/27 cases. And in case of each toxemic type, type takes type H again of subfrequent pregnancies at the rate of 4/7 cases, type AOH also repeats type AOH next time at the rate of 6/11 cases and type AH shown type H next time at the rate of 4/8 cases.

Summary and Conclusions.

1) Primary toxemia of late pregnancy occurs mostly in primipara. The distribution of each type of primary toxemia in primipara is unlike in multipara.

2) We can not put the primary toxemia in multipara and mild attack of secondary toxemia in the same category. The greater part of primary toxemia of multipara appear in bi-gravida and two thirds of them are accompanied by edema.

3) In primary toxemia type A is liable to appear in young primipara and type O in elderly primipara.

4) Pregnancy toxemias are liable to recur; 54.9% in primary toxemia and 67.7% in secondary toxemia habe had appearance of toxemia at next pregnancy.

5) The secondary toxemia is remarkably abundant in type H.

6) There is no specific relation between delivery interval and rate of second attack.

7) In our case, the rate of transition into the chronic type from pregnancy toxemia is 15.6%, and from primary toxemia it is only 3.5%, but from secondary toxemia it is 24.0%.

8) Chronic toxemia is always accompanied by hypertension and has always repeated pregnancy toxemia next time.

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Clinical Study on the Toxemia of late Pregnancy, with Special Reference to Its Recurrent Conditions, Transition to Chronic Type and Eclampsia.

2nd Report.

Clinical Study of Eclampsia and its Recurrence and Transition to Chronic Type.

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Zangemeister[1] classified hydrops gravidae in 4 stadiums, i.e. first stadium slight edema, second stadium edema at high grade, third stadium rise of blood pressure and increase of quantity of albuminuria and further in fourth stadium it shows cerebral symptoms, outbreak of eclampsia or severe cerebral symptoms without convulsion. Bar[2] and Essen-Moeller[3] called these severe symptoms on and after 3rd stadium as pre-eclampsia. And many physicians think at present yet that it develops from edema gravidarum into nephropathia gravidarum, further into pre-eclampsia and then into eclampsia. However, Bulicenko[4] does not approve any relation between renal disease and eclampsia, and also De Snoo[5] insists that there is no parallelism between the severity of the toxemia and the outbreak of eclampsia; Kuji[6] and Kushima sustain this opinion, too, and assert that eclampsia often complicates with nephropathia gravidarum; nevertheless, it does not always occur only in severe case, but it often complicates with a slight toxemia. But, in which type of toxemias and in what degree does eclampsia occur? About this problem, there have been very few investigations hitherto. So we have observed the relations between eclampsia and its clinical progress, and especially toxemic symptoms, and besides between renal symptoms and prognosis about 120 cases of eclampsia which were admitted in our clinic during 13 years (between 1932–1944). Here we report the following results.

Frequency of Eclampsia

Of 10.048 cases of hospital deliveries in our Dept. during 13 years 120

cases were eclampsia and it is equivalent to 1.2%. When we compare this result with other authors', it is equal to Nagai's[7] reprot (between 1922 and 1932) in our Dept., and also equal to Umezawa's[8] (Tokyo University) 1.12%, and Obata's[9] (Tokyo) 1.2%. But it is higher than Ono's[10] nation-wide statistics (0.89%) and Kuji's report (0.56% between 1931 and 1941, 0.84% between 1922 and 1930). It may due to the fact that eclamptic patients visit our Dept. a little more than abovementioned hospitals. Primigravidous eclampsia occupies 84 cases out of 3,668 cases of primigravidous women, and is equivalent to 2.3%. Multigravidous eclampsia occupies 36 cases out of 6,380 cases, equivalent to 0.6%. It is four times as many in primigravida and even in multigravidae one third of them is second gravidae. So it is worthy of notice that the eclampsia occurs mostly in primi-bi- or tri- gravida, and seldom in multigravida.

Relation with age: Hitherto it has been said that elderly primigravida is apt to cause the outbreak of eclampsia, but in our case in primigravida eclampsia occurs most frequently at the rate of 3.4% between 20 and 24 years of age, and those between 25 and 29 years of age is 1.6%, and over 30 years of age it is rather rare (0.2%). In multigravida, eclampsia occurs in over 40 years of age at the rate of 1.2% and it is somewhat high rate, but in other ages it shows between 0.4%–0.7%. So we do not admit any specific difference with age in multigravida.

Toxemic Symptoms on the Outbreak of Eclampisa.

The complication rate of eclampsia in the toxemia of late pregnancy.

There were 2,160 toxemic cases during our investigation term, in which the 120 cases of eclampsia were observed, i.e. toxemia complicates with eclampsia at the rate of 5.4%. In the reports of Young, Sym and Crowe[11] they say that eclampsia attacks toxemic patients at the rate of 6–7% in England. In our case, as it is shown in Table I, primigravidous toxemia is attacked about 4 times more than multigravidous toxemia.

Frequnancy of eclampsia classified by the toxemic types.

At the rate of 80% out of 116 cases (except on 4 obscure cases) eclampsia complicates with type AOH or type AH, so it has been hitherto considered that eclampsia is mostly originated from pre-eclampsia. Table I shows us the outbreak rate of eclampsia classified by the toxemic types. Yet even in the cases of slight toxemic symptoms we found the complication of eclampsia at the rate of 0.2%. Among both primi- and multigravida we can find no case of eclampsia complicated with type O. This is a proof for the denial of the theory of Zangemeister.[12] However, in 2 cases

TABLE I.

Frequency of Eclampsia Classified by the Types of Toxemia.

Gravida \ Toxemic types		A	O	H	AO	OH	AH	AOH	Slight	Obscure	Total
Primigravida	Number	4		4	3	6	18	42	3	4	84
	Frequency	5.0		2.0	8.3	9.2	19.1	34.1	0.2		11.1
Multigravida	Number	1		1	1		9	24			36
	Frequency	0.8		0.2	3.8		6.8	20.5			2.6
Total	Number	5		5	4	6	27	66	3	4	120
	Frequency	2.4		0.8	6.5	2.8	11.9	27.5	0.07		5.4

of type AO we have experienced that there had been type O just before the outbreak of eclampsia and with eclamptic attack they had become type AO. Therefore, such opinion can not be admitted that one is free from eclampsia because of being O type.

Kinds of ecalmpsia and types of toxemia.

Ecalmpsia gravidarum is mostly complicated with type AOH, primi and multi-gravida at the rate of 68% and 75% respectively, next to this, type AH at the rate of 18% and 25% respectively. Eclampsia parturientium is also mostly complicated with type AOH both in primi- (50%) and multi-gravida (79%) and next to this type AH at the rate of 27% and 14% respectively and in primigravida in addition to these types; though it is rare, eclampsia is further complicated with type AO, type A and type H. Among eclampsia puerperalis type AOH forms both in primi- and multigravida 28% and 40% respectively, and type AH forms each 11% and 40%. Further in primigravida, type OH forms 22% and type A 17%, and in multigravida type AO forms 10%. Type H both in primi- and multigravida forms 11% and 10% respectively.

Relations with seasons.

There are many cases of toxemia of late pregnancy in spring (24.7%) and in summer (25.0%) and somewhat less in winter (21.6%) and the least in autumn (14.9%). Inversely in the case of eclampsia they occur the most in autumn (1.5%), then in spring (1.3%), in winter (1.2%) and the least in summer (0.8%).

Eclampsia complicated with type AOH is decidely of great number in

autumn (46.5%), next in summer (36.4%), in winter (27.6%) and in spring (15.9%). Type AH is somewhat frequent in winter (16.7%), but there is no remarkable difference (10.2–12.1%) among other seasons. It is generally ascribed to the much perspiration in summer that many cases of eclampsia in this season are not complicated with edema. But as we find the fact that type O occurs most frequently in summer, we can not conclude so carelessly.

Immediate maternal mortality of eclampsia.

Maternal mortality is 10.8%. In primigravidous eclampsia it is 13.1% and type AOH (5/42 cases) has almost equal mortality with type AH (2/18 cases); also type OH has one death out of 6 cases and type A one out of 4 cases. In multigravidous eclampsia it is only 5.6% and that type AH alone has 2 deaths out of 9 cases. The degree of various toxemic symotoms except on eclampsia do not always agree with maternal prognosis. However, in eclampsia gravidarum maternal mortality is 20.6%, in eclampsia parturientium it is 10.3% and as for eclampsia puerperium no death has been reported.

Following pregnancies of eclamptic patients.

Hitherto we have not found so many reports which had investigated the following pregnant courses of eclamptic patients and for the most part many authors adopt the percentage not only with subsequent pregnancy, but also with one's repeated pregnancies; so it is not always correct results. For instance, Kjelland-Mordre[13] found the recurrence of toxemia at the rate of 57.8% in 52 times following pregnancies of 39 eclamptic patients; Chesley and Somers[14] at the rate of 47%; Dieckmann and Brown[15] at the rate of 40%. Menczer[16] reports the nephropathia gravidarum at the rate of 31.6% and eclampsismus at 10.5% out of 38 times following pregnancies of 24 eclamptic patients, and Mergner[17] found the recurrence of toxemia at the rate of 16% out of 187 times pregnancies of 106 eclamptic patients. As for recurrence of eclampsia Nevermann[18] reports 4 cases out of 60 cases, 11% by Bund,[19] 10% by Dieckmann and Brown and 5.3% by Mergner. As the symptoms at recurrent times Nevermann gives many hydrops gravidarum and Breakey[20] says that it is much more likely to complicate with albuminuria in subsequent pregnancies after eclampsia than in normal cases.

We have investigated the following pregnancies of 24 eclampsia, and among them recurrence of eclampsia occurred only in one case, which took place after primigravidous eclampsia. So we can say that eclampsia recurs scarcely. The recurrence of toxemic symptoms at subsequent pregnancies

is 14 cases out of 23 cases (60.4%). In primigravida it is 57.9% (11/19 cases) and in multigravida 75.0% (3/4 cases). And this is a little higher than the recurrent rate of non-convulsive toxemia of pregnancy which were previously investigated by us. 5 cases out of 24 cases (20.8%) remain toxemic symptoms even in pureperium for a long time and take chronic progress, i.e. 3 out of 19 cases (15.8%) are in primigravidous eclampsia and 2 out of 5 cases (40.0%) are in multigravidous eclampsia. And this rate is also higher than that of nonconvulsive toxemia of pregnancy.

TABLE II.

Recurrence Rate and Transition Rate to Chronic Type in Eclampsia Separated by Toxemic Types.
(Case Number and %.)

	Gravida \ Toxemic type	A	H	AH	AOH	Total
Recurrence rate	Primi gravida	1/2 50%	1/1 100%	4/7 57.1%	5/9 55.5%	11/19 57.9%
	Multi gravida				3/4 75.0%	3/4 75.0%
	Total	1/2 50%	1/1 100%	4/7 57.1%	8/13 61.5%	14/23 60.4%
Rate of chronic type	Primi gravida	0/2	0/1	2/7 28.5%	1/9 11.1%	3/19 15.8%
	Multi gravida				2/5 40%	2/5 40%
	Total	0/2	0/1	2/7 28.5%	3/14 21.4%	5/24 20.8%

The relation between the toxemic types on the outbreak of eclampsia and recurrent or chronic types is shown on Table II. Accordingly recurrent and chronic types mostly occur in eclampsia with type AOH or type AH; besides even in eclampsia with type A and type H occur recurrently, but they do not turn into chronic.

When we compare the toxemic types on the outbreak of eclampsia with the types of its recurrent cases, we find that one eclamptic case of A type changes into toxemia of O type at subsequent pregnancy, one eclamptic case of H type into toxemia of type H, 5 eclamptic cases of type AH into one toxemic case of type A, type H and type OH and 2 cases of type AOH respectively. And 12 eclamptic cases of type AOH into one case of type A, type AO and type AOH and 3 cases of type AH and 6 cases of type H respectively.

Accordingly, in case of eclampsia, this relation is similar to the case of non-convulsive toxemia, that is, toxemic types of subsequent pregnancies show various types. And out of 19 cases of second attack 9 cases include albuminuria, 15 cases have hypertension and 8 cases belong to type H, and chronic types always have hypertension. These facts are also agreeable with non-convulsive toxemia.

Table III.

Recurrence Rate and Transition Rate to Chronic Type Separated by Kinds of Eclampsia. (Case Number and %)

Gravida \ Kinds of eclampsia	Recurrence rate			Transition rate to chronic type		
	Ecl. gravida.	Ecl. parturi.	Ecl. puerp.	Ecl. gravida.	Ecl. perturi.	Ecl. puerp.
Primi gravida	3/6 50%	6/9 66.7%	2/4 50%	0/6	3/9 33.3%	0/4
Multi gravida	0	2/2 100%	1/2 50%	0	1/2 50%	1/3 33.3%
Total	3/6 50%	8/11 72.7%	3/6 50%	0/6	4/11 36.3%	1/7 14.3%

Relation between the kinds of eclampsia and recurrence and transition to chronic type of toxemia is shown on Table III. i.e. the recurrence rate of toxemia makes no great difference with the kind of eclampsia; but it is a remarkable fact, though the reason is not clear, that eclampsia gravidarum does not turn into chronic type. The toxemic recurrence at subsequent pregnancy seems to be abundant in the patients who were attacked by eclampsia on under 24 or over 30 years of age, and the older the patients are, the more is the chronic type.

Summary and Conclusions

When we compare the nonconvulsive toxemia which was described in 1st report with toxemia with eclampsia described in 2nd report, we get following conclusions;

1) Primary toxemia of late pregnancy occurs mostly in primigravidae both in eclampsia and in non-eclampsia.

2) About 80% of the eclamptic patients indicate usually either type AOH or type AH; on the contrary in case of the non-convulsive toxemia these types occupy only 23% and type A, type O and type H are comparatively of great number. In short, in case of nonconvulsive toxemia they take various types.

3) In case of younger primigravida in under 25 years of age who shows either type AOH or type AH, she has the greatest probability to occur eclampsia.

4) The second attack of toxemia at subsequent pregnancy is 60.4% in case of eclampsia, but in non-convulsive toxemia it is 54.9%. There is no great differences between them.

5) The secondary toxemia of non-convulsive cases is remarkably abundant in type H, but in case of eclampsia it has various toxemic types.

6) In primary and secondary toxemias, we may say that the maternal damage occurs scarcely though the symptoms are very severe, so far as they do not complicate with eclampsia, or do not turn into chronic type.

7) Transition rate to the chronic type in non-convulsive toxemia is 15.6% and in eclampsia it is 20.8%, and when observed in types which turn into chronic type the former is 27.9% (57/204 cases) and the latter is 23.8% (5/21 cases.)

8) Chronic type is always accompanied by hypertension and further more mostly has significant edema and albuminuria, and such a chronic type leaves various damages to mothers-bodies after delivery.

9) In multigravida especially in elderly multigravida, those who present toxemic symptoms, have already turned into chronic type or turn into chronic type in future, so we must pay special attention to them.

10) Generally the transition rate to chronic type increases with age.

I tender my greatful thanks to Prof. T. Shinoda and also to Ass. Prof. K. Kushima for valuable advices.

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On the Chemotherapy of Carcinoma. II.

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Chemotherapy of cancer is no doubt a world wide problem. In a preliminary[1] report last year, I concluded that chemotherapy of human cancer would be quite possible, delivering some cases of stomach cancer, which was cured by citronellal and citral intake, both remedies being unsaturated aldehydes of terpene series with C_{10}. As I mentioned in the previous report, the hint to this therapy has been gotten from the experiments of Strong.[2],[3]

The first case I tried citronellal was a man of 52 years with a child's head large bronchogenic cancer in the chest. After a few months betterment he got fluid in the thoracic cavity and disappointed left the hospital. But the experience in this case gave me a hopeful promise to the chemotherapy of cancer. This case was observed during October 1944–February 1945. Since then I treated many cases of cancer patient with citronellal and later also with citral. The remedies were diluted with cod liver oil and given per os and per rectum. Since last year I succeeded to make emulsion of the remedy which was injected intramuscularly. The emulsion can be prepared by the aid of some emulsifying chemicals. But we use at present NA type magnectostriction vibration (Nukiyama-Aoyagi), which is practiced by favour of the Tohoku University Electrical Communication Laboratory. Following cases were treated with success.

Y.M. 51 *Y. Male. A public official.* From Jan. 25 to June 1., 1945 in the clinic. Diagnosis: Stomach Cancer.

No malignant tumor in the family history. 6 years ago hyperacidity. Otherwise no remarkable disease. Moderately in tobacco and in vacco.

Present disease: Since last spring belching and pain in the stomach without direct connection with meal.

Status presens: Big stature, good constitution. Very meagre and anemic. Somewhat cachectic. Conjunctive and lip faded. Tongue coated. No palpable lymph gland of the neck and axilla. Lung and heart no change.

Abdomen flat. Abdominal wall soft. A tumor smaller than a hen's egg was palpated in the left epigastrium, hard, uneven. Not so well movable. Liver, spleen, kidney not palpable. Extremities thin.

Urine: normal. Faeces: no parasite eggs. Occult blood reaction positive.

Stomach juice, normal acidity. Blood: erythrocytes 4.05 million. Hb 60%. (Sahli's method).

X-ray examination of the stomach revealed a disk like niche of about 5.5 cm length diameter (Fig. 1 c.). The tumor was palpated coincided with this niche.

Therapy was started on Febr. 9, 1945 and continued till his leaving the hospital on Jul. 2. citronellal-cod-liver-oil āā 3.0 cc. per os and 8 cc. per anum. The condition of the patient was improved rapidly. After 2 months the tumor and niche were disappeared thoroughly. He gained 4½ kg of weight in 3 months and a half. Blood count 3.60 million erythrocytes and 58% Hb at the start of the therapy on February 11 and 4.84 million erythrocytes and 85% Hb. at discharge from the hospital. Since then about 4 years. Always healthy. (Fig. 1. a. b. c. d.)

D. M. 56 *Y. Male.* A government official. December 9, '47–May 2. '48 in the clinic. Diagnosis: Stomach cancer.

Family history: no cancer. He was hitherto very healthy. Malaria like fever in 13th and 25th ages. He drinks occassionally and smokes moderately. 7 months ago dull pain in the epigastrium 2–3 hours after meal, which lasted for some weeks. Since 2 weeks again the pain and heartburn. He lost rapidly the weight.

Status praesens: good constitution. Somewhat emaciated and anemic. Coated tongue. No palpable lymph gland on the neck and axilla.

Thorax: normal. Abdomen: flat. A tumor as large as a hen's egg in the epigastrium, hard, uneven.

Urine: normal. Stool: no parasites eggs. Occult blood reaction positive. Stomach juice slight hyperacidity.

X-ray examination revealed a niche as large as a sparrow's egg in the epigastruium, around which the tumour was palpated.

Therapy: Citronellal-cod-liver-oil āā with 1% citral, 3 cc per os and 8 cc per anum. After a month tumor and niche disappeared.

Blood count 3.71 erychrocytes and Hb. 71%. in admission and the same at discharge. During 10 months be continued to be healthy. Re-examination in March 1949, good health. X-ray finding of the stomach almost normal. (Fig. 2. a. b. c.)

E. S. 55 *Y. Male. Farmer.* July 5.–Oct. 2, '48 in the clinic. Diagnosis: Stomach cancer.

In his family no cancer. Bronchitis in 17th year. Otherwise always healthy. Since end of last year he felt heavy in the epigastrium, had dull pain in the stomach and became rapidly thin.

Status praesens: good constitution. Subcutaneous fat somewhat reduced. Somewhat anemic. Tongue coated. No palpable lymph gland on the neck and axilla.

Thorax: normal.

Abdomen: flat. A tumor as large as a hen's egg in the epigastrium a little on the left from the median line, hard and uneven, moderately movable.

Urine: normal.

Faeces: no parasites eggs. Occult blood reaction positive.

Stomach juice: slight hyperacidity.

X-ray revealed a large shadow defect in the lesser curvature of the stomach, where the tumor was palpated. No pain on palpation.

Therapy. Citronellal-cod-liver-oil $\overline{aa}+2\%$ citral, 3 cc per os and 8 cc per anum, later combined with injection of emulsion of citral (5%).

Tumor has rapidly diminished in its size. After a months treatment it was no more palpable. X-ray revealed slight stiffness of the lesser curvature. After 3 months the stomach mould was almost normal. Stomach uice hyperacidity. Blood examination

	July 6	Sept. 29
Erytyrocytes	4.15 mill.	5.17 mill.
Hb.	90%.	102%.

6 months after discharge reexamined. He gained $3\frac{1}{2}$ kg in weight. All the examination revealed good health. (Fig. 3. a. b. c.)

K. Y. 51 *Y. Male. Farmer.* July 9–Oct. 2, '48. Diagnosis: Stomach cancer.

No malignant tumour in his family. Hitherto very healthy. Drinks and smokes moderately.

Since last July he had heaviness in the epigasterium and diarrhoea occasionally. Since March of this year he had pain in the stomach, lassitude and rapid loss of body weight.

Status praesens: Good constitution. Somewhat meagre. Anemic. A few lymph glands of the size of soya bean on the left axilla.

Thorax: normal.

Abdomen: flat. A tumor larger than a hen's egg in the epigastrium, hard and uneven. Not so movable. No pain on palpation. Liver, spleen, kidney not palpable.

Urin: normal.

Stool: parasites eggs not found. Occult blood positive.

Gastric juice: Achylia.

X-ray revealed shadow defect in the pyloric part where the tumour was palpated.

Treatment. Citronellal-cod-liver-oil $\overline{aa}$+2% citral per os 3 cc, per anum 8 cc, later combined with injection of citral (5%) emulsion 3~8 cc.

Tumor diminished rapidly in the size and after three months only resistance in its place. Body weight and blood count about the same in admittance and at the discharge. A half year after discharge reexamination, the stomach almost normal.

These are the cases, whose diagnosis were we think mostly exact. But following cases are examples of uncertain diagnosis. K. S. 61 years old man, who had a skirrhus of the stomach in the X-ray examination, achylia and moderate cachexy, was cured by citronellal. A man of 52 year (A. Ch,) admitted in the hospital under diagnosis of primary hepatic cancer, whose liver being enlarged to the navel, had his liver diminished day by day under the action of citronellal. He was discharged after 3 months and was in good condition, when be visited us one year later. Cases which was improved transitorily by this therapy are numerous. Two cases of bronchogenic carcinom, lost their cancer cells in the sputum and in the pleural fluid by this treatment. (Fig. 4. a. b. c.). In one case of stomach cancer with abdominal fluid and a case of a very large retroperitoneal sarcoma with peritoneal fluid the tumor cells of the fluid disappeared in the course of this therapy. (Fig. 5. a. b. c. d.). Abdominal fluid in the latter case was no more to get after repeated puncture. But we could not save them to our great regret.

Some cases of esophageal carcinoma, cardia carcinoma and pyloric carcinoma, which were quite advanced and the cancer tissue infiltrated almost all the circumference of the wall and destroyed the tissue so far as to the tunica muscularis, were quite improved, but in the course of few months, again increased stenosis, very probably owing to the shrinkage of the scar tissue which might have replased the cancer tissue. Only not much advanced cases gave good results.

From all these we can conclude that most of the stomach cancer would be cured by this therapy, if we could begin with proper stage.

It would be better to consider in this place surgical treatment of cancer in connection with the chemotherapy. I don't think this therapy should prevent the cancer patient from surgical operation. On the contrary it would widen indication of operation of cancer patients. I report here a case of a stomach cancer inoperable at first because of its extent and adhesion, but which was operated with good result after a period of this therapy.

T. S. 50 *Y. Male.* An employee of a company. Jan. 16–March 26

'48 in the clinic and then operated. Diagnosis: Stomach cancer. Since 10 years he had stomach pain and heartburning. These 2 months he became rapidly thin. Vomited almost every evening. Status praesens: Very thin and cachectic. Diffuse resistance in the epigastrium and a rugged irregular shaped tumor as large as a goose egg was palpated, immovable. X-ray revealed shadow defect in the pyloric part extending on both curvatures, corresponding the tumor.

Gastric juice: achylia. Citronellal-cod-liver-oil with 2% citral per os (3 cc) and per anum (8 cc).

Tumor has diminished and appetite increased but transitorily.

The second X-ray showed a shadow defect localised in the pyloric part and tumor became movable but passage of the pylorus became worse. Operation by Prof. Muto. The tumor was resected. But the surgeon noted that recidiv would be inevitable. On reexamination after 10 months, he was found in a very good condition. Recurrence was not detected. (Fig. 6 a. b. c. d.)

The following case of cancer in the S Romanum was first operated and anastomosis was set and then chemotherapy.

S. H. 49 *Y. House-wife.* Admitted on Nov. 5, 1948, operated on Nov. 9, 1948. Father died from rectum cancer. She had habitual constipation because of hemorrhoid from which she suffered and used to apply laxativa. Before 3 months she had pain in the abdomen and diarrhoea after sea-bathing. Since then she had often diarrhoea and following constipation by slight nutritional disturbance. Lately she had obstinate constipation. Laxative and klysma were of no use. She had heaviness in abdomen and borborygma. She became very thin. Extensive tympany in abdomen. Digital palpation in rectum and romanoscopy examination gave nothing. X-ray following barium meal and klysma revealed a high sitted stenosis in the S romanum. Operation showed a skirrhus of colon sigmoideum about 50 cm from anus. As the tumor infiltrated retroperitoneal tissue, radical operation was impossible. An antastomosis of colon sygmoidea of upper and lower part of the stenosis was made. Then she was treated by us with citral and citronellal. She gained rapidly in weight, 11½ kg in 2½ months. No complaint in abdomen. After discharge on March 18, 1949 she continued to get fatter. Now 6 months after begin of chemotherapy she gained more than 16 kg. (Fig. 7. a. b. c.).

Recently I have known that Boyland and associates[4] resumed the work of Strong. They treated the spontaneous mamma carcinoma of mouse and grafted Crocker sarcoma with many sorts of aldehydes. Among those aldehydes Boyland mentioned citral was quite efficacious. So far as I could read I don't know whether there are reports in which human cancer was treated with aldehydes.

So far as our experiments on chemotherapy of the grafted "Yoshida sarcoma" with aldehydes concerns, which is now only malignant tumour of laboratory animal available in Japan, good effect of citral, citronellal and oenanthol is obvious. A part of these experiments was reported in the Japanese Cancer Society at Osaka on April 6, 1948.

SUMMARY.

In this paper I have reported four cases of stomach cancer, who had been cured with chemotherapy and on reexamination after 6 months to 4 years were found free from relapse. Some other cases, in whom chemotherapy was efficacious, are given briefly. Two cancer cases, who were treated with chemotherapy under cooperation of surgery, are mentioned.

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Explanation of figures:

Fig. 1. Y.M. 51 Y. male. Stomach cancer.
- a. Jan. 26. 1945. Before treatment.
- b. June. 1. 1945. After treatment.
- c. Febr. 1. 1945. Before treatment (X-ray photo. Natural size).
- d. May 29. 1945. After treatment. (X-ray photo.)

Fig. 2. D.M. 52 Y. male. Stomach cancer.
- a. Dec. 9. 1947. Before treatment. (X-ray photo.)
- b. Jan. 27. 1948. During treatment. (X-ray photo.)
- c. March 9. 1948. After treatment. (X-ray photo.)

Fig. 3. E.S. 55 Y. male, Stomach cancer.
- a. June 29. 1948. Before treatment. (X-ray photo.)
- b. July 30. 1948. During treatment. (X-ray photo.)
- c. September 21. 1948. End of treatment. (X-ray photo.)

Fig. 4. T.O. 52 Y. male.
Bronchogenic cancer with pleural effusion.
- a. April 30. 1947. Just before treatment. (Micro-photo.)
- b. May 16. 1947. During treatment. (Micro-photo.)
- c. June 3. 1948. After 2 months' treatment. (Micro-photo.)

Fig. 5. D.S. 23 Y. male.
Retroperitonealsarcoma with peritoneal fluid.
- a. Aug. 13. 1948. Photo on admission.
- b. Aug. 13. 1948. Before treatment. (Micro-photo.)
- c. Sept. 14. 1948. During treatment. (Micro-photo.)
- d. Sept. 22. 1948. No tumor cell was seen. (Micro-photo.)

Fig. 6. T.S. 50 Y. male. Stomach cancer.
- a. Jan. 28. 1948. Before treatment.
- b. March 9. 1949. 10 Months after operation.
- c. Dec. 19. 1947. Before chemotherapy. (X-ray photo.)
- d. March 26. After 2¼ months' chemotherapy. (X-ray photo.)

Fig. 7. S.H. 49 Y. female. Cancer of S romanum.
- a. Nov. 9. 1948. Before anastomosis and before chemotherapy.
- b. June 3. 1949. After anastomosis and after 3 months of chemotherapy.
- c. Sight of operation.

Fig. 1 a

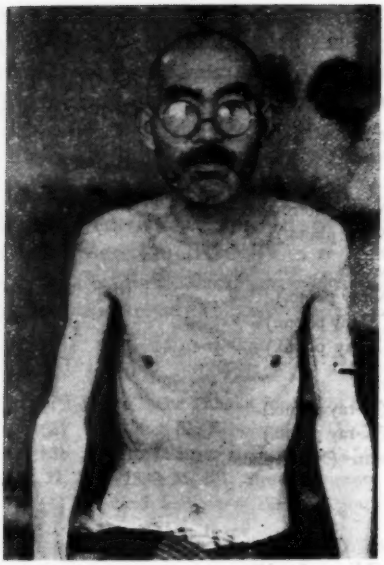

Fig. 1 b

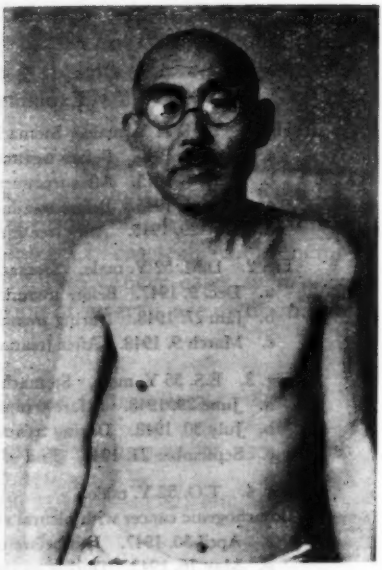

Fig. 1 c.

Fig. 1 d.

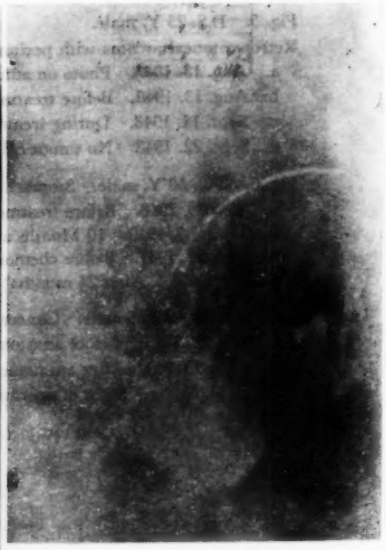

Fig. 2 a.

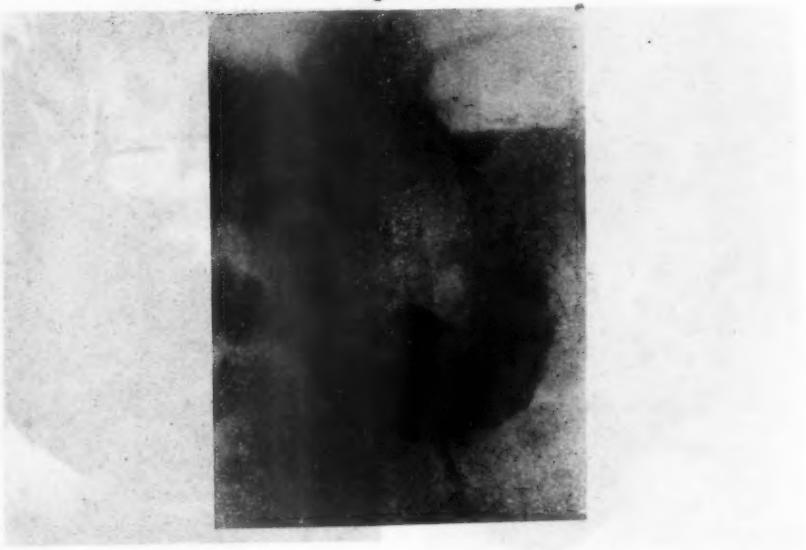

Fig. 2 b.

Fig. 2 c.

Fig. 3 a.

Fig. 3 b.

Fig. 3 c.

Fig. 4 a

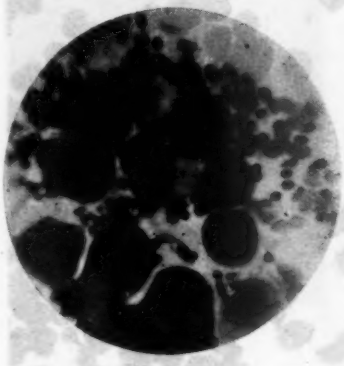

Fig. 4 b.

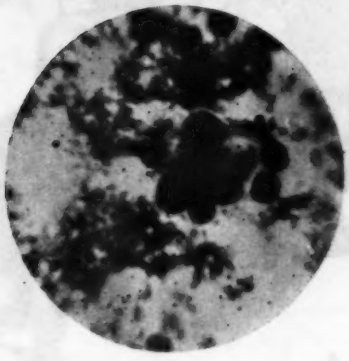

Fig. 4 c.

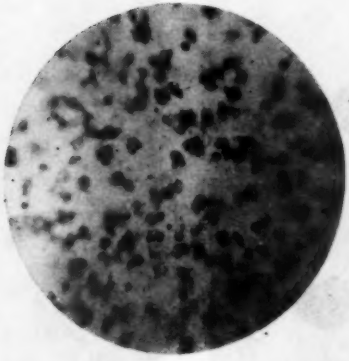

Fig. 5 a.

Fig. 5 b.

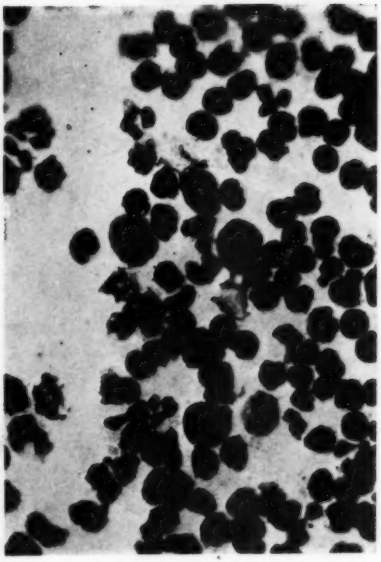

Fig. 5 c.

Fig. 5 d.

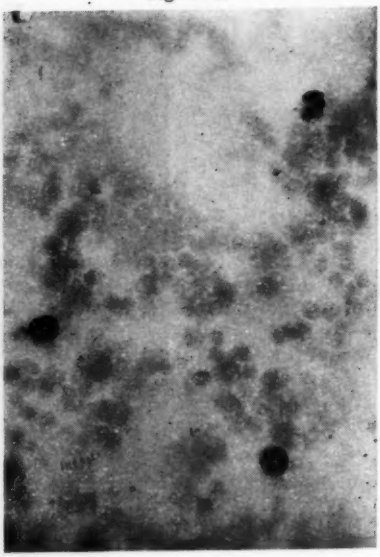

Fig. 6 a.

Fig. 6 b.

Fig. 6 c.

Fig. 6 c.

Fig. 7 a.

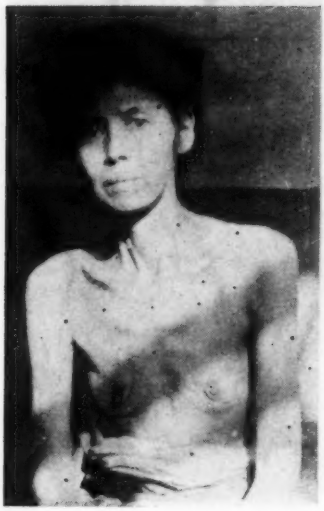

Fig. 7 b.

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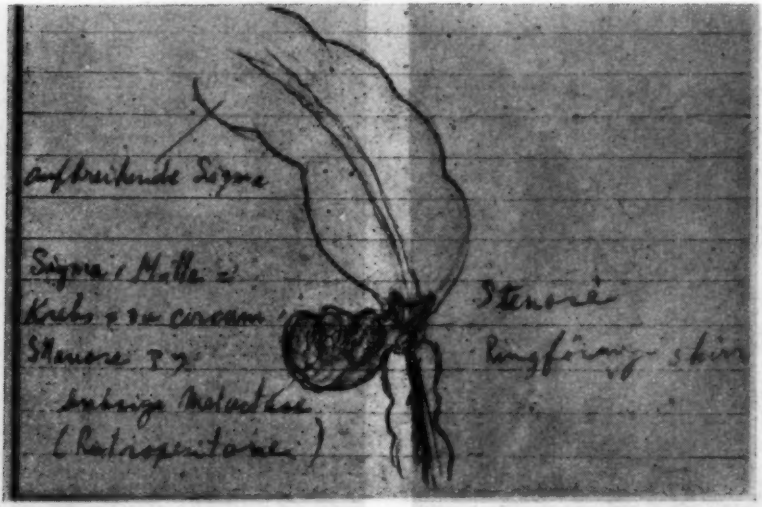

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Electrophysiological Studies of Color-Blindness.

By

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It was shown by Motokawa[1)2)3)] that the electrical excitability of the eye recovers after a brief illumination of the eye along a time course characteristic of the wave-length of the light used for illumination. This phenomenon has hitherto been studied extensively on normal man,[5)] but only tentatively on color-blind subjects.[4)] In this paper, therefore, we have made more detailed observations on several anomalous trichromats and dichromats.

Experimental.

Method.

The arrangement for electrical stimulation of the eye is shown schematically in Fig. 1, in which K_1 and K_2 represent the contacts of a spring rheotome. The stimulating current was a constant current pulse of 100 msec. which was produced by opening the contacts K_1 and K_2 in succession. The stimulating voltage was applied to the subject's eye through a pair of silver electrodes of 2×1.5 cm^2 in size, one of which was placed above the eyebrow and the other on the homolateral temple of the subject. The electrical contact between the electrode and skin was secured by electrode paste rich in NaCl.

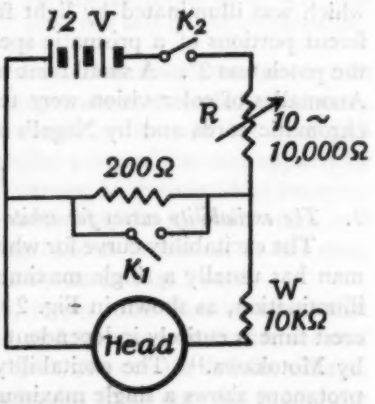

Fig. 1. Electrical circuit for stimulation of the eye. W is a resistance to reduce the effect of variations in the resistance of tissue.

The experiment was carried out in a dark room. After dark adaptation of about 20 minutes, the electrical threshold was measured in such a way that the stimulating voltage which was high enough to elicit a distinct electrical

phosphene, was reduced step by step until the subject could no more distinguish the stimulus in question from one far below the threshold. The gradation of the stimulating voltage was made by means of the variable resistance R, which was varied at first roughly, later in smaller steps, for example, in steps of 50 to 20 ohms towards the threshold. The value of the resistance R was found to be about 2,000 ohms at the electrical threshold of normal man, though it showed individual as well as daily variations. The conditions of skin seemed to be an important factor for the absolute value of threshold.

In the next place, the electrical threshold at a certain definite moment, say 0.5, 1, 2, 3 seconds etc. after the end of illumination lasting two seconds was determined in a similar manner as stated above, and thereafter the threshold without pre-illumination was measured again to be sure that the state of the subject remained unaltered during the experiment. The pause between successive stimulations was about 20 seconds in these experiments.

In order to express quantitatively the effect of illumination on the electrical excitability after illumination, a quantity ζ was introduced which is defined as follows: $\zeta=\frac{E_t-E_0}{E_0}\times 100$, where E_0 represents the electrical excitability (the reciprocal of the threshold) without pre-illumination, and E_t the excitability at the moment t seconds after pre-illumination. The curve obtained by plotting ζ as ordinate against the time after illumination as abscissa is called the "excitability curve."

For the pre-illumination, a circular patch of ground glass was used, which was illuminated by light from an incandescent lamp or from different portions of a prismatic spectrum. The visual angle subtended by the patch was 2°. A small faint red light served to fix the eye in the dark. Anomalies of color vision were tested as usual by Ishihara's pseudo-isochromatic cards and by Nagel's anomaloscope.

Results.

1. The excitability curves for white light of Normal and the color-blind subjects.

The excitability curve for white light obtained from the fovea of normal man has usually a single maximum at about 2 seconds after the end of illumination, as shown in Fig. 2 (a). The time to the maximum or the crest time is entirely independent of the intensity of illumination, as shown by Motokawa.[1] The excitability curve for white light obtained from a protanope shows a single maximum like that of normal man, whereas the curve taken from a deuteranope consists of two distinct elevations, as illustrated in Fig. 2 (b) and (c). It is of great significance that there is such a conspicuous difference in the form of the excitability curve between the

two kinds of color-blindness. This difference is practically important, because it enables us to distinguish very easily protanopia from deuteranopia. Apart from such practical importance, it will serve greatly for elucidation of the retinal mechanism of color-blindness.

However, in this respect, the data obtained from anomalous trichromats are more instructive. In Fig. 3, excitability curves for white light taken from three cases of deuteranomalia are illustrated. As can be seen in this figure, the above two curves show three elevations, which may, for simplicity, be denoted by R, G and B. The elevation R has the shortest crest time of all the elevations, but it can be seen that the absolute value of this crest time varies from case to case; in the case (a), the crest time of the R elevation is 0.75 seconds, while in the other two cases it is 1.5 seconds. The B elevation which has the longest crest time shows little individual variations with regard to the crest time; it is about 4.3 seconds in all cases illustrated in this figure. The most remarkable feature of the G elevation which has the intermediate crest time is that its magnitude is always smaller than the magnitude of the other elevations. The magnitude of the G elevation is so small in the case (c) of Fig. 3 that it cannot be seen at all. The excitability curve of this case apparently consists of only two elevations so that it cannot be distinguished from the excitability curve of a deuteranope. Nevertheless, the method which will be described in section 3 could reveal the hidden G process in this case, but could not in the case of deuteranope shown in Fig. 2 (c).

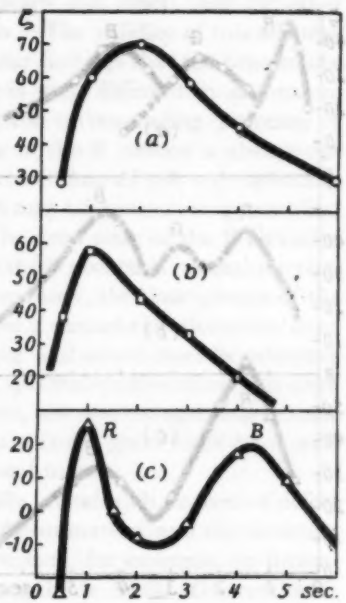

Fig. 2. Excitability curves for white light obtained from normal man (a), from protanope (b) and from deuteranope (c). Ordinates: percentage increases in electrical excitability of the eye. Abscissæ: time after the end of illumination.

As shown in the curve (a) of Fig. 3, it was sometimes observed that two different thresholds appeared for one and the same time after the end of pre-illumination. In such cases, the subject, at a certain definite voltage, felt it very difficult to distinguish the stimulus from one far below the threshold, but on further decreasing the voltage, discrimination became easier, and thus the true threshold was reached. The ζ-value evaluated from the true threshold naturally represented a point on one elevation, and

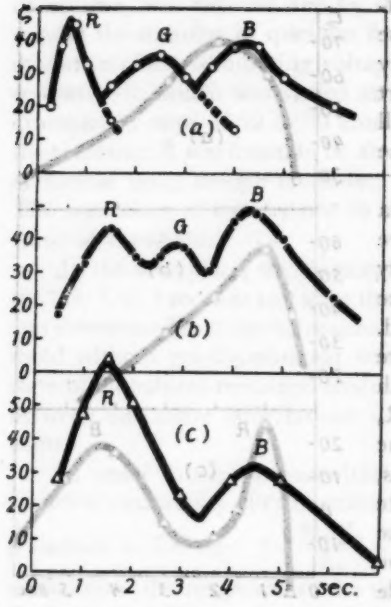

Fig. 3. Excitability curves for white light taken from three deuteranomalous subjects.

it was found that the ζ-value evaluated from the relative threshold found on the way to the true threshold fell on the extension of the neighboring elevation. This fact indicates that the three humps of the excitability curve represent independent different processes, but not different phases of one and the same process. Such double thresholds mentioned above could be found only when the stimulating voltage was graded in sufficiently small steps and the subject was careful enough to find an abrupt change in the strength of the electrical phosphene. Otherwise, we would obtain the envelope of the three elevations as the excitability curve.

In view of this situation, it is not difficult to understand that the excitability curve consists of a smooth envelope having a single maximum, when the G elevation is sufficiently high. Thus, we can ascribe the simple form of the excitability curve for white light obtained from normal man to the dominance of the G elevation.

As will be shown later, the G process underlying the G elevation has its preferential region in the green part of the spectrum. Therefore, we can make the G process correspond to the G component of the trichromatic theory. The theory advocates that the G component develops only slightly in deuteranomalous persons and not at all in deuteranopes. And, our findings that the G elevation is found always smaller than the other elevations in the deuteranomalous and cannot be found in the deuteranope, fits very satisfactorily into the scheme of the trichromatic theory.

The theory assumes the lack of the R component in the protanope. The lack of the R elevation, however, will not cause such double humps on the excitability curve, for the remaining elevations G and B appear overlapping so that the envelope will constitute a single maximum. In reality, the excitability curve taken from the protanope consists of a single maximum, as shown in Fig. 2 (b). On the basis of our findings, we can see the validity of the assumption of the trichromatic theory that the component R or G is absent in dichromats, and shows insufficient development in anomalous

trichromats. It has been assumed by König[6] and others that the other two components are normal in color-blinds. The validity of this assumption, however, is very doubtfull in view of our findings that the time course of the elevations of the excitability curve is very different in anomalous trichromats and dichromats from that of the corresponding processes in normal man. For example, the crest time of the R process is always one second in normal subjects, whereas the crest time of the corresponding elevation takes various values between 0.75 and 1.5 seconds in green-blind and incompletely green-blind subjects. The crest time of the B elevation is about 4.3 seconds in these subjects, while the B process in normal man has the crest time of about 3 seconds. In this manner, the time courses of the basic color processes in color anomalies show a remarkable deviation from normal. However, the assumption of König and others must be admitted to a certain extent, especially so long as the spectral sensitivity of each component is concerned: As will be shown later, the wave-length dependence of the color processes of color-blinds shows a fairly good agreement with that of the corresponding processes in normal man.

Anomalous trichromatic vision generally includes all degrees of defect between normal color vision and complete dichromatism, and this situation is very clearly exemplified in our phenomenon; for example, in incompletely green-blind persons, the relative magnitude of the G elevation is greatly different from case to case even under one and the same experimental conditions. This feature of the phenomenon is of great practical importance, for the relative magnitude of the G elevation can be utilized as a quantitative measure for deuteranomalia. But it must be borne in mind in practice that this measure depends on various factors such as the intensity of illumination, the size of test objects etc. In the following section, the effect of these factors will be discussed.

2. The dependence of the Retinal color processes on the intensity of illumination

In general, anomalous trichromats require a longer time for the appreciation of colors, and a higher intensity and larger visual angle before the hue can be discriminated.

Our experiments show that the G elevation of the excitability curves of deuteranomalous subjects disappears on reducing the intensity or the size of illumination. This fact indicates that the G process is more sensitive to reduction of visual stimulation than the other processes. It deserves attention that such a tendency is not restricted to color anomalies, but can be found also in normal subjects; normal man becomes incompletely green-blind when the intensity or size of illumination is sufficiently reduced. Even at a high intensity and large size, the parafoveal region of the retina of normal man is incompletely green-blind. This can be

shown objectively by the fact that the excitability curve for white light taken from a parafoveal region of normal man shows distinct three maxima, as reported by Motokawa.[5] This must be due to the insufficient development of the G process in this region of the retina.

A series of systematic measurements at the fovea of normal man demonstrated that the excitability curve for white light suffers a systematic deformation by reducing the intensity of the white light in such a way that the excitability curve becomes more and more flat with decreasing intensities. This fact is worthy of note, for it makes a striking contrast to the well-established fact that the shape of the excitability curve for monochromatic light is almost independent of its intensity.[1][5] And, such a deformation of the excitability curve for white light may be ascribed to the greater sensitivity of the G process to the reduction of visual stimulation. To make the evidence for this consideration more complete, the dependence of each component process in the excitability curve for white light was investigated separately in the following way. The G process can be measured selectively by fixing the interval between the end of pre-illumination and the electrical stimulation at two seconds, because it can be shown in various ways that the maximum of the G process is attained in two seconds, and that this process is the dominant process in the fovea of normal man. The R process can be measured selectively by fixing the interval at one second, and the B process by fixing the interval at three seconds, because we know that the crest times of the R and the B process are one and three seconds respectively. As to the determination of the

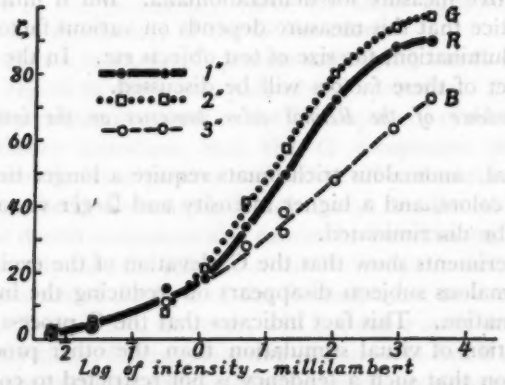

Fig. 4. Dependence of the magnitude of the three proceses R, G and B in normal man on the intensity of white light used for pre-illumination. Ordinates: percentage increases of electrical excitability of the eye at one sec. for the process R, at two sec. for the G and at three sec. for the B after the end of illumination.

crest time of each component process, see the preceding paper by Motokawa.[4])

In Fig. 4 the dependence of each component process on the intensity of illumination is illustrated, which is given in logarithmic units in this figure. As can be seen in this figure, the magnitude of the G process is greater at higher intensities of illumination than that of any other process, and the smallest one is the B process under this condition. But, as the intensity decreases, the three curves converge on a single common curve, representing that the G process suffers the greatest reduction by lowering the intensity of illumination. This tendency is far stronger in deuteranomalous subjects than in normal man. Hence they give excitability curves consisting of two elevations under unfavorable conditions of illumination.

3. The dependence of the retinal color processes on the wave-lengths of spectral lights.

We have confirmed in the above experiments that there are three basic color processes in the fovea of trichromats. We must now determine the proportions in which the three component processes are provoked by colored light of any wave-length, for our sensation of color must be determined by these proportions, as the theories of color vision suggest. Such proportions have hitherto been determined by troublesome experiments of color mixing, and the data obtained from these experiments are represented as the so-called excitation curves, in which the stimulus value for each primary color is plotted as ordinate against the wave-length of spectral light as abscissa. In our experiments, the magnitude of each basic process can be determined directly by the procedure outlined above. If we map out the ζ-value for the entire range of the spectrum, we shall obtain a curve which will, for simplicity, be termed "the spectral locus" in the following.

In order to determine the spectral locus of the R process, the interval between the end of pre-illumination and the electrical stimulus must be fixed at one second which corresponds to the crest time of the R process in normal man. Starting at the red end of the spectrum, the wave-length of the pre-illuminating light is varied step by step, and at each wave-length the ζ-value at the moment just one second after cessation of the light-stimulus is determined. In this way the curve R in Fig. 5 (a) was obtained. In a similar way, the spectral loci of the G and the B process can be determined, fixing the interval between the light-stimulus and the electrical one at two and three seconds respectively. The spectral loci determined in this way are shown as the curves G and B in the same figure. These curves show how the three components are excited by spectral light of any wave-length in normal man.

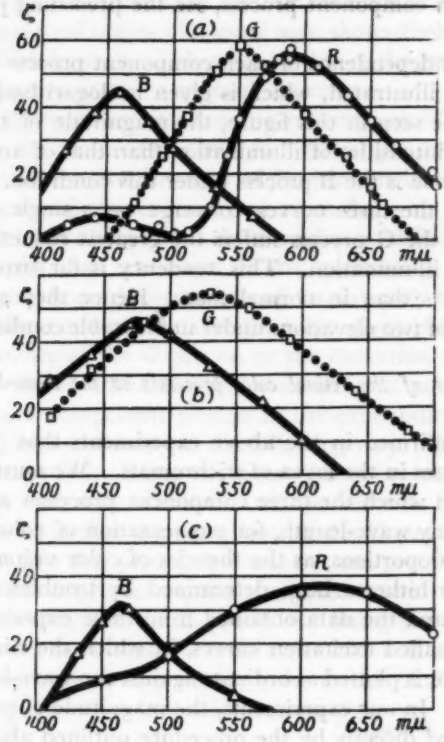

Fig. 5. Wave-length dependence or spectral locus of each color process determined at the fovea of normal man (a), of protanope (b) and of deuteranope (c).

In (c) of the same figure are illustrated the spectral loci R and B which were obtained from a deuteranope by a similar procedure. In this case, the interval between the light-stimulus and the electrical stimulus was fixed at one second for the R locus and at four seconds for the B locus, because the crest times of the R and the B elevation were one and four seconds respectively in this subject.

The two loci G and B in (b) of Fig. 5 were obtained from the protanope whose excitability curve for white light is shown in Fig. 2 (b). The interval between the optic stimulus and the electric one was in this case one second for the G locus and 2.5 seconds for the B locus respectively, because the crest time of the excitability curve for green light was found to be one second, and that of the excitability curve for blue light was 2.5 seconds in this subject. It is obvious that the absolute value of the crest time of each

process has little significance for identification of the processes in different individuals. The identification of the processes is possible only by taking their spectral properties into account. Comparing (a), (b) and (c) with one another, it can be seen that the spectral loci equally labelled have their maxima nearly in the same region of the spetrum: The B locus of the normal subject has its maximum at about 460 mμ, that of the protanope at about 470 mμ, and that of the deuteranope at about 460mμ. Such a satisfactory agreement in their spectral properties justifies to use the same notation for these processes inspite of such different values of their crest times as 3 sec. in the normal subject, 2.5 sec. in the protanope and 4 sec. in the deuteranope.

The maximum of the G locus of the protanope is located at 530mμ, while that of the normal subject lies at 550mμ. There is a slight discrepancy also between the R loci of the normal subject and of the deuteranope: The locus R is located at about 590mμ in normal, and at about 610mμ in the deuteranope, and these differences are too great to be regarded as the error of measurements. They are surely not due to the error of measurements, because the G and R loci determined in a parafoveal region of the retina of the same normal subject showed a close agreement with the corresponding loci of the dichromats with respect to the location of their maxima. It is, therefore, probable that the color processes in the dichromats have the same spectral properties as the corresponding processes in the parafoveal region of normal man.

In the protanope, we could by no means find any process having the same spectral properties as the R process; in a series of experiments carried out fixing the interval between the optic and the electric stimulus at values other than those adopted in the experiment stated above, the locus obtained always ran parallel with either of the two loci G and B, but no locus having the maximum near 600 mμ could be obtained. Similarly failed all our attempts to find any process which could be identified with the G process in the case (c).

Fig. 6 shows the spectral loci of the deuteranomalous subjects whose excitability curves are shown in Fig. 3. As the intervals between the optic and the electric stimulus the crest times of the three elevations were chosen. In the case (c) which showed only two elevations in the excitability curve, the same interval as in the case (b) was adopted for dertermining the G locus. As can be seen in this figure, the locations of the maxima of the loci B and R coincide within the limits of error with those of the corresponding loci of normal man, showing that the behavior of these two processes is normal so far as the spectral properties are concerned. The locus G is, on the contrary, very anomalous in all cases not only in its maximum height but also in its extent over the spectrum. It is worthy of note that there

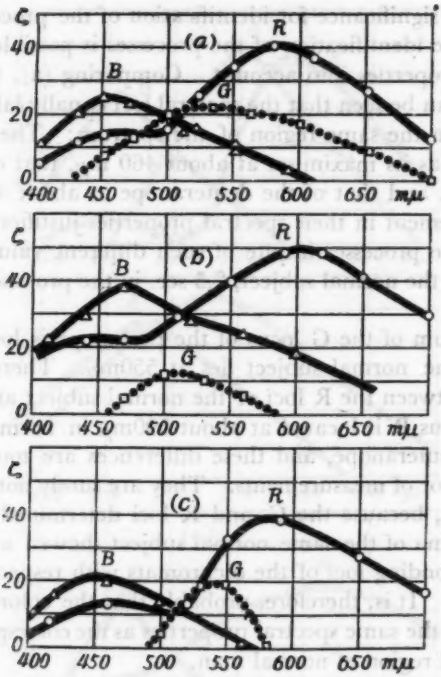

Fig. 6. Spectral loci of the three color processes determined at the fovea of three deuteranomalous subjects.

are striking individual differences with regard to the extent of the G locus: For example, in the case (a), it extends over the spectral range from about 700 to 440mμ, whereas the locus is limited within such a narrow range as between 570 and 490mμ in the case (c).

It seems to us that the ability of hue discrimination depends greatly upon the extent of the G locus in deuteranomalous subjects, for the defect in color vision was found to be the mildest in the case (a) in which the G locus was of nearly normal extent, and the defect was most severe in the case (c) which showed the narrowest locus. As a matter of fact, the excitability curve for white light obtained from this subject consisted of two elevations and could not be distinguished from that of a deuteranope. If we relied only upon the routine tests of color-blindness we should have taken this case for a true deuteranope.

Summary.

The time course of changes in electrical excitability of the eye after

2 seconds' illumination by white light was investigated in normal and color-blind subjects.

1. The excitability curve obtained by plotting ζ-values or percentage increases in electrical excitability as ordinates against the time after the end of illumination as abscissae showed a single maximum at 2 seconds in normal subjects. In the deuteranope, two distinct elevations were found on the excitability curve, while the curve taken from the protanope showed a single maximum at one second.

2. The excitability curve for white light obtained from deuteranomalous subjects showed distinct three elevations which were termed R, G and B in the order of rapidity of development. It was shown that the three elevations represent three independent processes but not different phases of one and the same process. The development of the G process is weak in the deuteranomalous and entirely lacking in the deuteranope, so that there appear only two elevations in the excitability curve of the deuteranope.

3. The G elevation of the excitability curve taken from deuteranomalous subjects disappeared at a sufficiently low intensity of illumination, so that the excitability curve taken under such conditions could not be distinguished from that of the deuteranope.

4. The wave-length dependence or spectral locus of each color process was investigated. The locus of the B process has its maximum at about 460mμ in all cases of normal and color-blinds. The R locus could not be found in the protanope, and the G locus was found lacking in the deuteranope. The R and G loci obtained from the color-blinds had a maximum at 610 mμ and 530 mμ respectively. The locations of these maxima did not coincide precisely with those of the corresponding loci taken from the fovea of normal man, but showed a close agreement with those of the corresponding loci taken from a parafoveal region of the retina of normal man.

In the deuteranomalous, the B and R loci showed normal behavior in all respects, but the G locus was found always smaller than normal not only in the maximum height but also in the extent of the locus over the spectrum. In one case of the deuteranomalous, the G process extended over such a narrow range of the spectrum as between 490mμ and 570mμ. It is worthy of note that the defect in color vision was so severe in this case that it could not be distinguished from a true deuteranope by the routine anomaloscopic test and by our method of excitability curves for white light.

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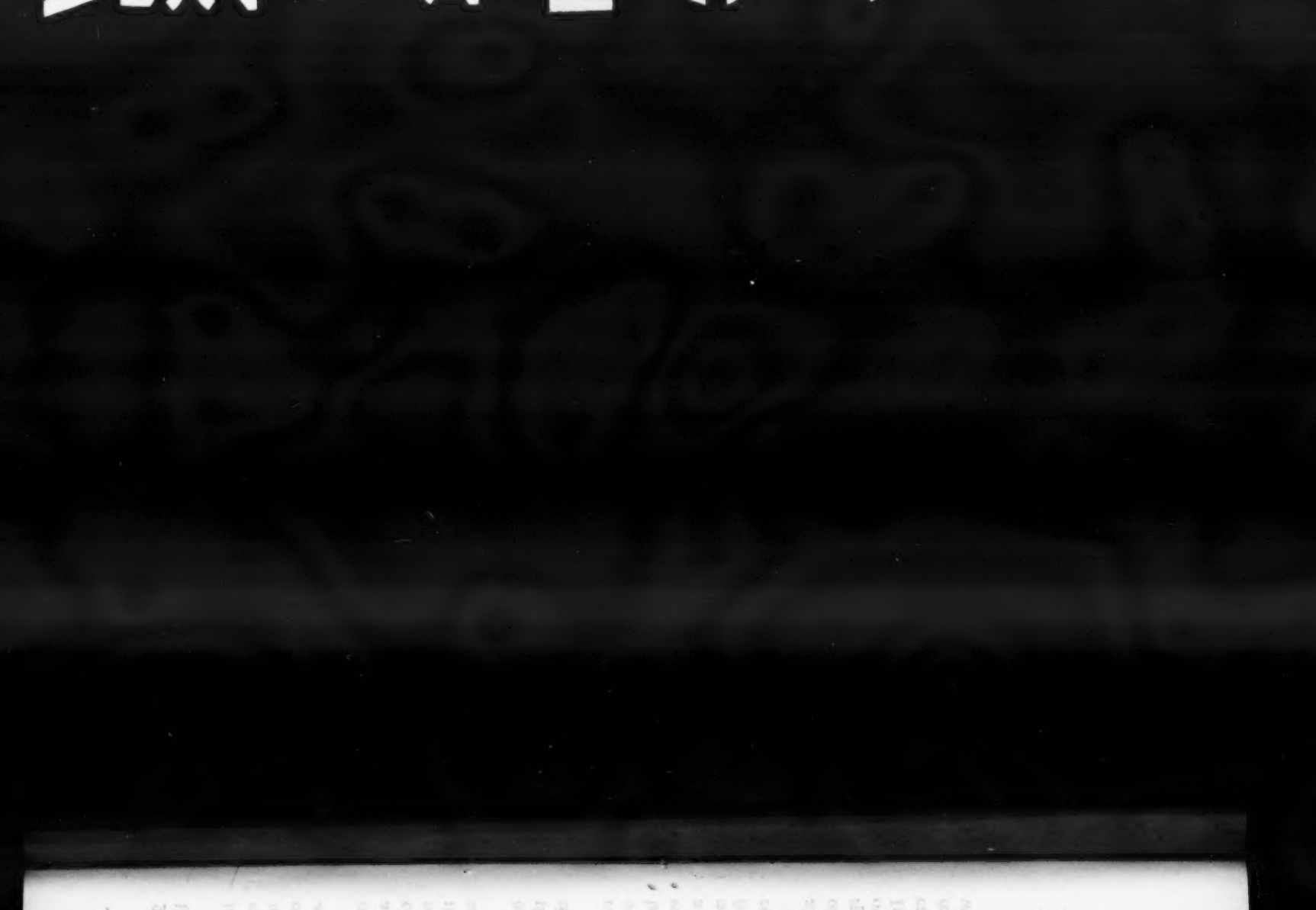
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